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ANNALS  
OF  
TROPICAL MEDICINE AND  
PARASITOLOGY

ISSUED BY THE  
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

PATRON : HIS MAJESTY THE KING

Edited by

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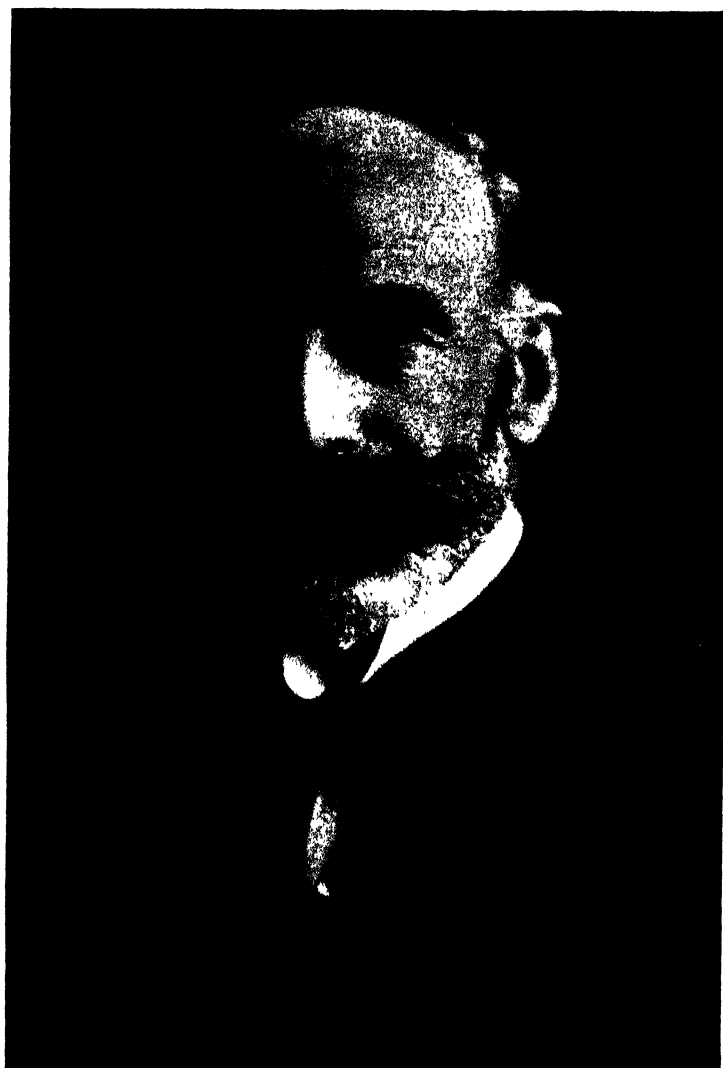
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*Robert Newstead*

## IN MEMORIAM

The editors record with deep regret the death on February 16th, 1947, of Emeritus Professor ROBERT NEWSTEAD, M.Sc., F.R.S., at the age of 87 years.

Newstead was appointed Lecturer in Economic Entomology and Parasitology to the Liverpool School of Tropical Medicine in 1905, and was elected the first holder of the Dutton Memorial Chair of Entomology on its foundation in 1911. He took part in the School's expeditions to Jamaica in 1908 to investigate the prevalence of sarcoptic mange among cattle, and to Malta in 1910 to study the bionomics of sandflies. In 1911 he worked on the Royal Society's Sleeping Sickness Commission in Nyasaland, and from 1913 to 1914 he was a member of the Inter-Departmental Committee on the same disease. During the Great War of 1914-18 he was engaged by the War Office to take charge of investigations into the control of fly-borne diseases occurring in France and Flanders, and directed the Liverpool centre of investigation set up by the Royal Society Grain (Pests) War Commission.

Newstead retired from the Chair of Entomology in 1924, but continued his connection with the Liverpool School of Tropical Medicine as Director of the School's museum until 1929, when he was awarded the Mary Kingsley medal. From the time of his retirement until within a few months of his death, he was actively engaged upon work on the Roman occupation of Chester, a subject on which he was the leading authority.

Newstead's published works on entomology and archaeology form a considerable bulk of much importance, his *opera magna* being the 'Monograph of the Coccidae of the British Isles,' published by the Ray Society in 1900-2, and the 'Guide to the Study of Tsetse-Flies,' issued in 1924. He was an editor of these *Annals* from their inception in 1907 until December, 1929.



# POLYMORPHISM IN *TREPONEMA RECURRENTIS* AND *SPIROCHAETA VINCENTI*

BY

A. T. CULWICK

AND

H. FAIRBAIRN

(From Tinde Laboratory, Tanganyika Territory)

(Received for publication May 2nd, 1946)

The technique for determining the sign of the electric charge of trypanosomes by the examination of thin blood films (Fairbairn and Culwick, 1946) was applied to the spirochaete *Treponema recurrentis*, and the lengths of 774 of these organisms were measured.

The mean length of those apparently attracted by the red blood-cells (the positively charged variant) was  $25.94 \mu \pm 0.40 \mu$ , while that of those apparently repelled (the negatively charged variant) was  $23.69 \mu \pm 0.39 \mu$ —a difference of  $2.25 \mu \pm 0.56 \mu$ , which is statistically significant ( $P < 0.0001$ ) and shows that the differentiation employed was not an arbitrary one.

The length distributions of these two variants are shown in the histograms in fig. 1 and fig. 2. They are obviously not statistically normal distributions. It was found, however, that each histogram could be accurately fitted by the sum of three symmetrical distributions, curves no. 1–3 in fig. 1 and no. 4–6 in fig. 2, and an analysis of these curves showed that none departed significantly from a Gaussian distribution. The sums of each group of three curves are shown by the broken lines, and the detailed figures are given in Table I.

It will be seen that the negative and positive variants differ in composition. This difference is statistically significant ( $P < 0.05$ ), which again points to the differentiation employed between the two electrical variants not being an arbitrary one.

The evidence strongly suggests that *Treponema recurrentis* exists in three forms, a short, an intermediate and a long, each form occurring as two electrical variants, giving six forms in all. In this respect the spirochaete closely resembles *Trypanosoma rhodesiense*, which also exhibits six forms (Fairbairn and Culwick, 1946); and it is tentatively suggested that possibly *Treponema recurrentis* also undergoes a process of syngamy, and that its six forms have a similar significance to those of *T. rhodesiense*.

The lengths of 778 *Spirochaeta vincenti* from a throat-swab were next examined. The length-distribution curve, which departs markedly from normality, is shown in fig. 3, and is there compared with that of the total of all six forms of *T. recurrentis* drawn to a comparable scale. It will be seen that these two curves bear a striking resemblance to one another.

Owing to the absence of red blood-cells on the slide of *S. vincenti*, we could not say whether it occurred in this instance as one or two electrical variants. It was found, however, that its length-distribution curve could be dissected into three symmetrical curves (fig. 4 and Table II), and an analysis of these three curves showed that none



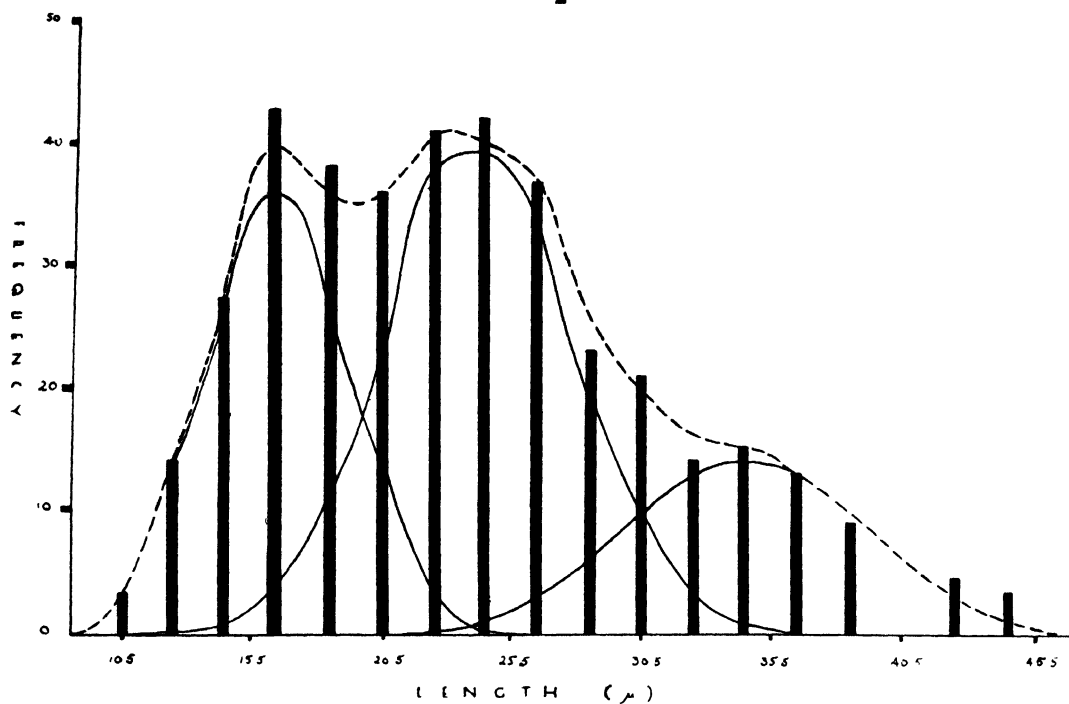


FIG. 1. Length distribution of negatively charged forms of *T. recurrentis*.

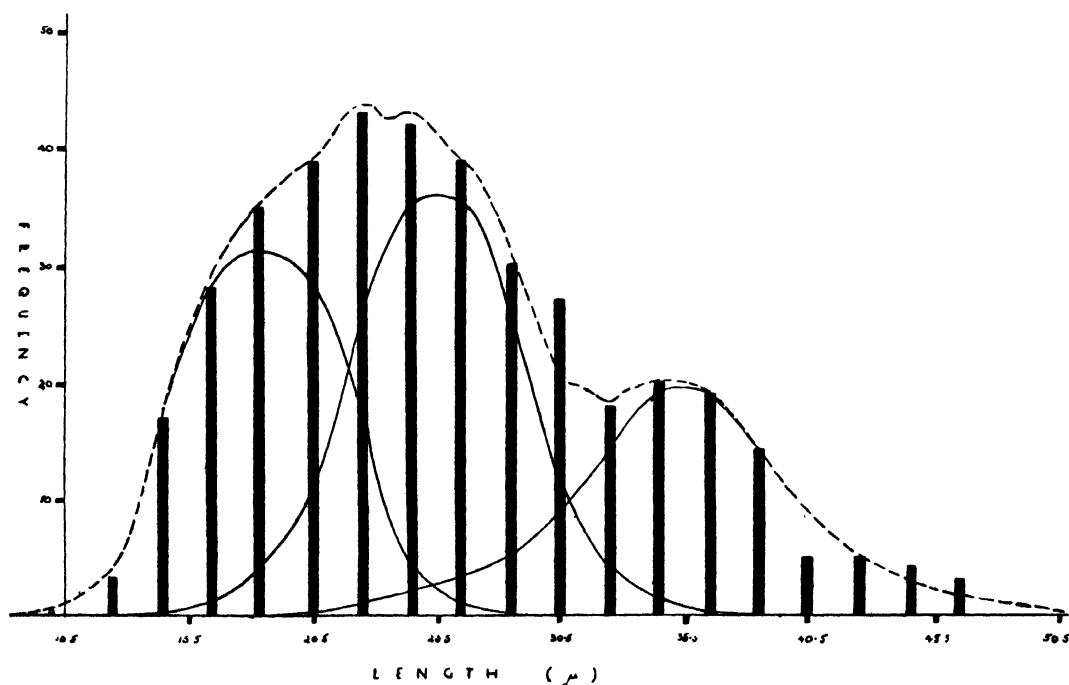


FIG. 2. Length distribution of positively charged forms of *T. recurrentis*.

TABLE I  
Length distribution and analysis of *T. recurrentis*

Length in $\mu$	Negative variant					Positive variant				
	Curves				Total found	Curves				Total found
	No. 1	No. 2	No. 3	1+2+3		No. 4	No. 5	No. 6	4+5+6	
10.5	3	—	—	3	3	1	—	—	1	—
12.5	14	—	—	14	14	4	—	—	4	3
14.5	25	1	—	26	27	17	—	—	17	17
16.5	36	4	—	40	43	28	1	—	29	28
18.5	25	11	—	36	38	31	4	—	35	35
20.5	14	22	—	36	36	28	11	—	39	39
22.5	3	38	—	41	41	17	26	1	44	43
24.5	—	39	1	40	42	4	35	2	41	42
26.5	—	34	3	37	37	1	35	3	39	39
28.5	—	20	6	26	23	—	26	5	31	30
30.5	—	10	10	20	21	—	11	9	20	27
32.5	—	3	13	16	14	—	4	14	18	18
34.5	—	1	14	15	15	—	1	19	20	20
36.5	—	—	13	13	13	—	—	19	19	19
38.5	—	—	10	10	9	—	—	14	14	14
40.5	—	—	6	6	—	—	—	9	9	5
42.5	—	—	3	3	4	—	—	5	5	5
44.5	—	—	1	1	3	—	—	3	3	4
46.5	—	—	—	—	—	—	—	2	2	3
48.5	—	—	—	—	—	—	—	1	1	—
Total ...	120	183	80	383	383	131	154	106	391	391
Mean ...	16.5	24.5	34.5	—	23.69	18.5	25.5	35.5	—	25.94
S.E. ...	—	—	—	—	$\pm 0.39$	—	—	—	—	$\pm 0.40$
$g_1$ ...	-0.48	-0.11	-0.43	—	—	0.00	-0.09	0.02	—	—
$\sigma_{g_1}$ ...	0.44	0.36	0.53	—	—	0.42	0.39	0.47	—	—
$\chi^2$ ...	2.12					5.46				
Composition (per cent.)	31	48	21	100	—	34	39	27	100	—

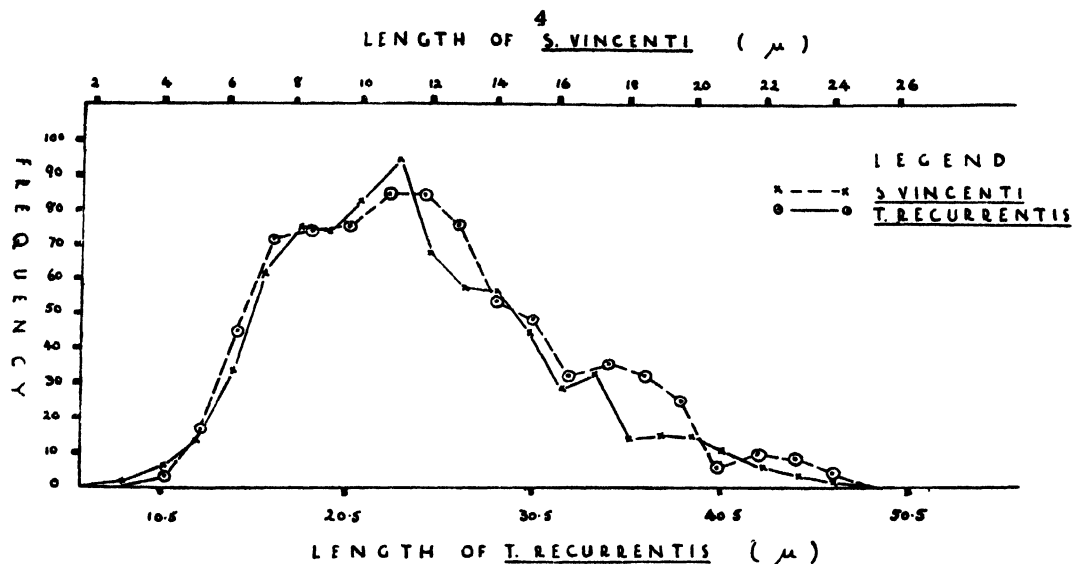


FIG. 3. The length distribution of *T. recurrentis* and *S. vincenti* compared.

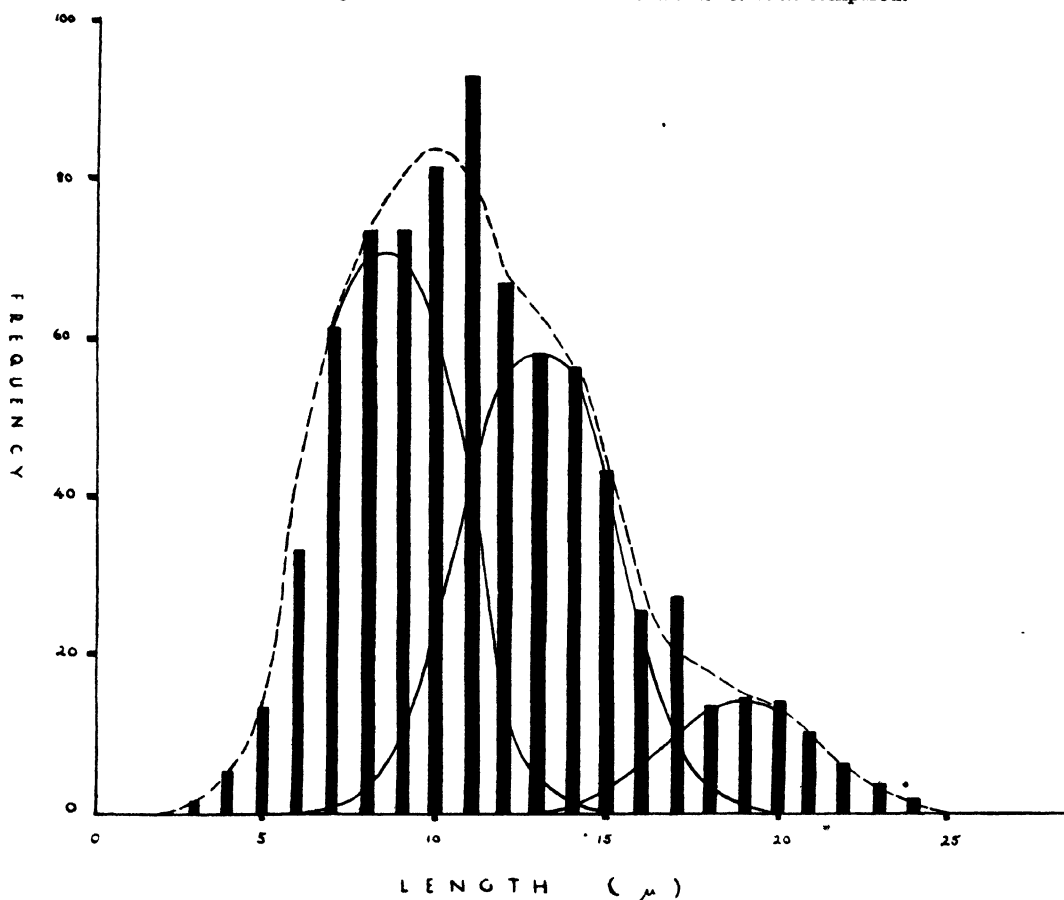


FIG. 4. Length distribution of *S. vincenti*.

departed significantly from a Gaussian distribution.  $g_1$  was zero by construction, while the three values of  $g_2$  all showed insignificant platykurtosis.

The data therefore point to *S. vincenti* also being a mixture of three main forms, though they do not enable us to decide in this case whether each of these forms is composed of a single type or whether it is an admixture of a positively and a negatively charged variant with their mean lengths close together, as in *T. recurrentis*.

TABLE II  
Length distribution and analysis of *S. vincenti*

Length in $\mu$	Curves				Total found
	No. 1	No. 2	No. 3	1+2+3	
3	1	—	—	1	1
4	5	—	—	5	5
5	13	—	—	13	13
6	40	—	—	40	33
7	61	—	—	61	61
8	70	3	—	73	73
9	70	10	—	80	73
10	61	23	—	84	81
11	40	40	—	80	93
12	13	55	—	68	67
13	5	58	—	63	58
14	1	55	—	56	56
15	—	40	3	43	43
16	—	23	6	29	29
17	—	10	10	20	31
18	—	3	13	16	13
19	—	—	14	14	14
20	—	—	13	13	14
21	—	—	10	10	10
22	—	—	6	6	6
23	—	—	3	3	3
24	—	—	—	—	1
Total	380	320	78	778	778
Mean	8.5	13	19	—	—
$g_1$	0.32	0.40	— 0.65	—	—
$\sigma g_2$	0.25	0.28	0.54	—	—
$\chi^2$	...	...	...	11.2	

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# DEVELOPMENTAL FORMS OF *TRYPANOSOMA BRUCEI* IN THE 'SALIVA' OF *GLOSSINA PALLIDIPE* AND *GLOSSINA AUSTENI*

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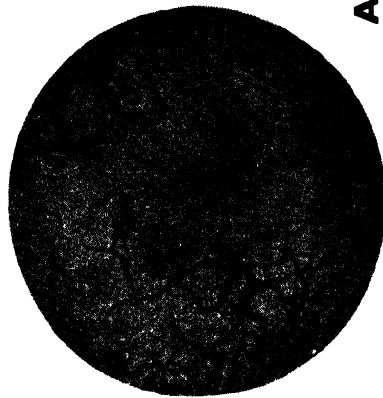
(From the Veterinary Research Laboratory, Kabete, Kenya Colony)

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In the course of experiments on the transmission of *Trypanosoma brucei* by the tsetse *Glossina pallidipes* and *G. austeni*, we have observed in the 'salivary' exudate of the flies a sequence of developing trypanosomes which explains, in greater detail than hitherto described, the changes that take place in the development of this trypanosome from the proventricular to the final infective stages. The sequence suggests modification of earlier conceptions of the life-cycle of *T. brucei*, particularly with regard to the derivation and nature of the forms which invade the salivary glands. Neither the proventricular trypanosomes nor the long slender crithidia invade the glands to develop directly into flagellates which give rise to metacyclic trypanosomes. Completion of the life-cycle in the fly appears to be dependent on the development of the proventricular trypanosomes into peculiar post-proventricular forms, which give rise, by unequal fission recorded and illustrated by Lloyd (1930) as unusual forms from the mid-gut of *G. tachinoides*, to the long slender crithidia of earlier workers and to short crithidia. Mature infection of the fly is determined by the ability of the short crithidia to reach, and to become established in, the salivary glands.

The tsetse-flies were reared from pupae collected in the coastal fly-areas of Kenya and from pupae deposited by laboratory-bred flies. They were fed every other day on animals reacting to a Mariakani-camel strain of *T. brucei* maintained at Kabete since September, 1944, and passaged through a variety of vertebrate hosts by bites of infected tsetse flies and by inoculation of infected blood. The flies were starved for 48 hours on about the 14th day after the first feed on a reacting animal. They were then induced to 'salivate' on a glass slide by a method somewhat similar to that adopted by Koch (1905), Bruce *et al.* (1914), Lloyd and Johnson (1924), but improved, to facilitate examination of large numbers, by Burt and Vanderplank (Vanderplank, 1944). Glass slides were warmed on a metal water-tank thermostatically controlled at 38–40° C. (100·4–104·0° F.). These slides were placed over a tube containing a single fly, which, in an attempt to probe the warm surface, deposited one or more drops of fluid—which collected at the tip of the proboscis—on to the slide. The fluid or exudate dried readily, and was immediately fixed in methyl alcohol and stained with buffered Giemsa. Subsequent to the first probe, the exudate from each fly was examined at regular intervals of 48 hours, and the fly was fed immediately afterwards. We were thus able to pick out, at an early stage, individual flies in which the trypanosomes were in the process of developing. The sequence of development was deduced from the successive appearance and the relative numbers of





**A**

A mass of long slender crithidia, together with dividing proventricular forms (centre). ( $\times 450$ .)



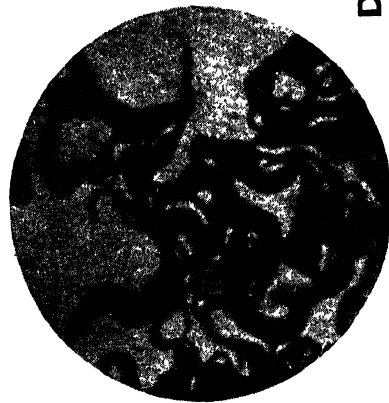
**B**

Post-proventricular forms, proventricular trypanosomes, long slender crithidia and daughter crithidia. ( $\times 850$ .)



**C**

Early granular metacyclics and one long slender crithidia. ( $\times 2,050$ .)



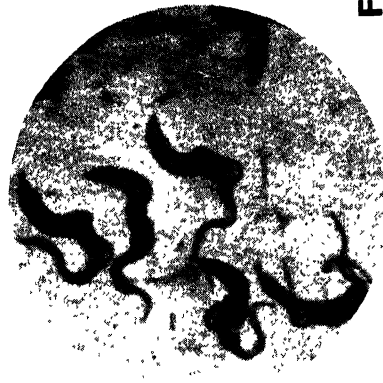
**D**

A mass of early metacyclics. ( $\times 2,050$ .)



**E**

Early metacyclics with long flagella. ( $\times 2,050$ .)



**F**

Intermediate forms of metacyclics. ( $\times 2,050$ .)

All forms as seen in the 'salivary' exudate.

the different forms, beginning with the proventricular trypanosomes with which in *T. gambiense*, according to Robertson (1913*b*), the gut development of the cycle culminates.

The first indication of the establishment, or at least of initial development, of *T. brucei* in a fly is the appearance in the exudate of a number of long trypanosomes referred to by earlier workers as the proventricular form (figs. 1–4). Their presence in the so-called ‘salivary’ exudate has been interpreted (Bruce *et al.*, 1914) as proof that the tsetse has the power of regurgitating the contents of its proventriculus and intestine together with the salivary secretion.

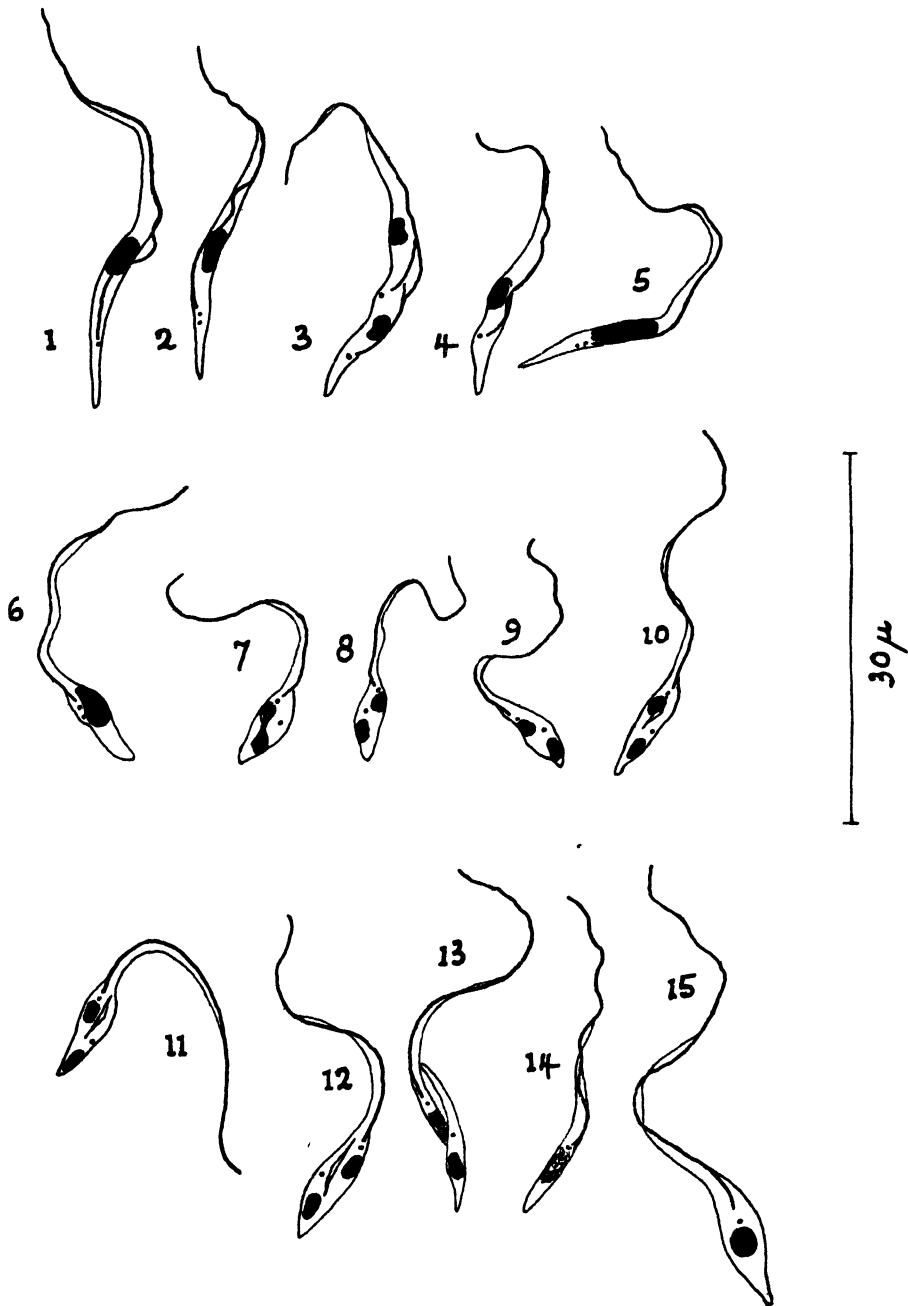
The flagellates in the exudate are in various stages of development. At first only a few are found, and they are often embedded in a densely stained matrix, which, as was noted also by Koch (1905), contains no blood-corpuscles. Their numbers increase rapidly; either at or following their first appearance they occur in such a tangled mass that it is difficult to determine their individual form and structure. Nevertheless, numerous isolated specimens were examined, and some were drawn by camera lucida. They are typically trypaniform, as in the case of *T. gambiense* (Kleine and Taute, 1911; Fraser and Duke, 1912; Robertson, 1913*b*; Lloyd and Johnson, 1924; and others). Fraser and Duke (1912) illustrate a similar form for *T. brucei* from the gut of *G. palpalis*, and Lloyd (1930) from *G. tachinoides*. Robertson (1913*b*) gives a drawing of such a form as a specimen newly arrived in the salivary gland of *G. palpalis*. The proventricular trypanosomes of *T. brucei* in *G. pallidipes* and *G. austeni* vary from  $34\mu$  to  $40\mu$  in length. The body tapers posteriorly to a long nearly pointed snout, and anteriorly to merge into the free flagellum. The greatest width varies from  $1.5\mu$  to  $2.0\mu$  where the oval or round and compact nucleus is situated at approximately half the length of the body. The kinetoplast is nearly always mid-way between the nucleus and the posterior end. Dividing forms occur, but are not frequent. Fission is preceded by the division of the kinetoplast into two, one of which appears always to remain at the original site, whereas the other moves forward. The final division, involving the nucleus and flagellum as well as the kinetoplast, is oblique or transverse—a feature observed by Robertson (1913*b*)—and is possibly a significant mode of multiplication to be contrasted to the longitudinal division of the long blood forms in the vertebrate host. The infrequency of these dividing proventricular trypanosomes in the exudate tends to support the view that their reproduction is largely confined to an earlier (gut) rather than to a later (proventriculus or salivary gland) phase of development.

The trypanosome of the proventricular type undergoes a completely different change, which, we are led to believe by events, is in the direct line of development of *T. brucei*. It is a process which, if it occurs in *T. gambiense*, appears to have escaped notice. Robertson (1913*a*) does not include it in the life-cycle, which she described and illustrated, of a trypanosome from British East Africa ‘obtained originally from a donkey which was supposed to have contracted the disease at a spot on the *safari* road from Ngobotok to Baringo’ (Duke, 1913), where *T. rhodesiense* or *T. gambiense* were unlikely to have existed. Lloyd (1930), however, records and illustrates what he calls some unusual forms met with in the mid-gut of *G. tachinoides*, but states that their significance, if any, is not understood.

The cytoplasm of proventricular trypanosomes about to undergo this change flows into the posterior portion of the flagellate. This part of the body gradually increases to form a prominent lanceolate or tadpole-like head (figs. 6 and 7), whereas the other part



of the body becomes more attenuated. These post-proventricular flagellates are from  $26\mu$  to  $44\mu$  long. The nucleus elongates and moves into the swollen posterior end, and

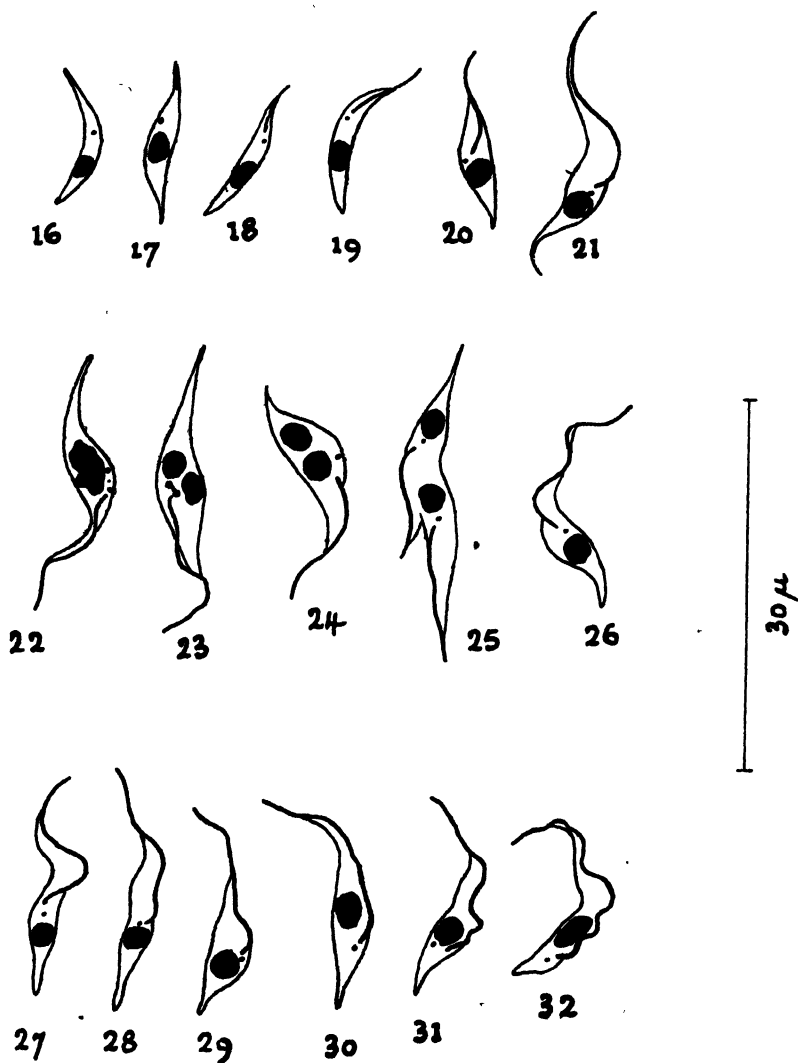


FIGS. 1-15. Proventricular trypanosomes and post-proventricular tadpole forms.

at the same time or soon afterwards divides into two. It is preceded by the division of the kinetoplast, also into two, each of which takes a position anterior to each of the nuclei. The anterior pair in particular is in close proximity, and to the kinetoplast is attached the flagellum (figs. 8 and 9). The cytoplasm between the pairs of nuclear bodies splits longitudinally to form two crithidia (figs. 10–13)—one a short aflagellate crithidia cut off as a section of the swollen end, and the other a long flagellate crithidia comprising the remaining section of the swollen end and extending anteriorly to incorporate the original attenuated body of the proventricular trypanosome. The latter crithidia appears to be the long slender form which, in the case of *T. gambiense*, Kleine and Taute (1911) referred to as 'male' and which Robertson (1913*b*) considered to be merely a degenerating slender form. We agree with Robertson on the ultimate fate of these slender crithidia, but there is some indication (fig. 4) that they may produce two or more short crithidia before final degeneration. They are thinner ( $0.75\text{--}1.25\mu$ ) than the proventricular trypanosomes, and their nucleus is long, with loosely arranged chromatin granules (fig. 14). The short ( $9\text{--}14\mu$ ) daughter crithidia, if they survive, develop flagella (figs. 16–20) after separation. We have been unable to find a flagellum in these short crithidia before separation, and we cannot explain Lloyd's finding that these crithidial forms at division have the flagellum sticking out at a right angle to the body, unless Lloyd's specimens represented an abnormal development as a result of the unfavourable conditions in the mid-gut. On the fate of these daughter crithidia depends the successful establishment or failure of infection of the salivary glands and the ultimate production of metacyclic trypanosomes. They are hardly ever abundant in the 'salivary' exudate, probably because they migrate readily to the salivary glands where they become attached; whereas the tangled mass of immature long flagellates are easily regurgitated in mass and are frequently present in larger numbers, probably representing an accumulation of excessive supplies from the gut. If, however, the production of daughter crithidia is adversely affected, further development in the salivary glands may be retarded or partially or wholly arrested. Under favourable conditions, these crithidia reach the salivary glands and attach themselves. Preliminary examination of some infected glands confirms observations made by earlier workers that attachment is effected by the flagellar or anterior end of the parasites, and that the free posterior extremity develops a short or long, hair-like, and apparently flexible, process (fig. 21). Some of these crithidia become detached, and appear as aberrant forms in the 'salivary' exudate. The majority (haptomonads) remain fixed and multiply (figs. 22–24). Multiplication, at this stage, is by equal or nearly equal fission. The kinetoplast and the nucleus divide and pair off in a crithidial arrangement. A flagellum arises from the kinetoplast of the distal half, which separates by a transverse or oblique split (fig. 25). If complete separation is delayed, the kinetoplast in the distal portion may begin to migrate to a position posterior to the nucleus and thus produce a transitional or trypaniform flagellate. Transitional forms do not appear in large numbers in the 'saliva,' and we have not yet ascertained whether or not some or all attach again to produce more haptomonad forms. It is clear, however, that the free flagellated crithidia (nectomonads) ultimately pass through a transitional phase (figs. 26–30) and develop into metacyclic trypanosomes (figs. 31 and 32).

The metacyclic trypanosomes vary considerably (groups 33 and 34). On their first appearance in the saliva the majority are broad ( $1.75\text{--}3.00\mu$ ). Later, thinner forms ( $1.25\text{--}2.00\mu$ ) become increasingly common. It seems as if there are two types of meta-

cyclic trypanosomes, or two stages of development, which, for convenience, we call the early (group 33) and the late (group 34) stages. Close examination, with measurements, reveals intermediate forms with regard to width and length, and other differences occur which might prove significant on statistical analyses. Many have no free flagellum. In the so-called early metacyclics the free flagellum (when present) ranges in length from

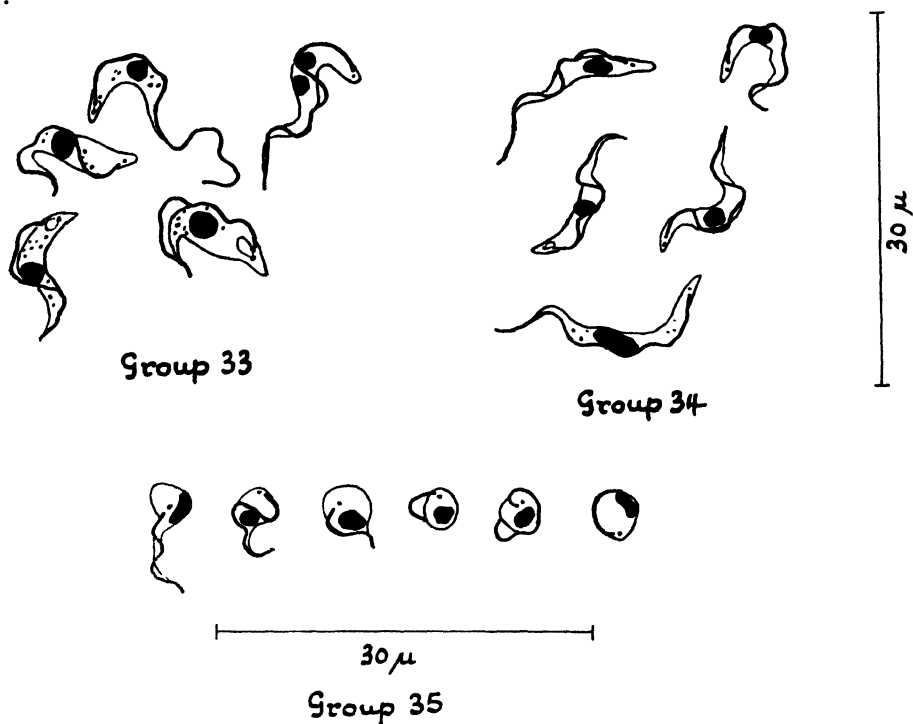


FIGS. 16-32. Daughter crithidia and transitional forms.

$1.0\mu$  or less to, in some instances, as much as  $8.0\mu$ . In the late metacyclics its range ( $1.0\mu$  or less to  $5.25\mu$ ) is shorter. Lloyd and Johnson (1924), incidentally, give the range as  $1.1-3.5\mu$  and make no reference to the aflagellate forms. Wenyon (1926), on the other hand, states that 'At all stages [of *T. brucei* and *T. gambiense*] the trypanosomes have flagella except the metacyclic forms, which resemble the short stumpy trypanosomes

occurring in the blood.' Other differences or variations concern the absence or presence of granular bodies in the cytoplasm and the shape of the nucleus, which is nearly always rounded in the early, and elongated in the late, metacyclic infective trypanosomes. Apparently, division takes place in both forms. We have seen metacyclics with two kinetoplasts and two nuclei, but we have not seen further evidence of splitting and separation. Furthermore, these forms are not common in the 'salivary' exudate.

Our evidence does not favour the inclusion of rounded forms in the life-cycle, as illustrated by Robertson (1913a). These coiled-up and rounded forms (group 35) appeared in the 'saliva' several days after the late metacyclic trypanosomes in the initial cycle and after several successful transmissions. They were rarely found in the 'saliva' until the infected flies showed signs of weakness, or when a fly was disinclined to feed regularly or was about to die. It seems to us, therefore, that these forms arise as a result of adverse conditions and are unessential to the completion of the normal cycle of development in the fly.



GROUPS 33-35. Early and late metacyclic trypanosomes and rounded forms.

Such is our interpretation of the sequence of development, from the proventricular to the final infective stage, of *T. brucei* in *G. pallidipes* and *G. austeni*. The progressive phase during the first invasion by the gut forms is later masked by subsequent reinforcements at intervals of one or two more days, when the large variety of flagellates tend to confuse the picture.

The 'salivary' exudate from some flies was taken, when possible, at intervals of 24 hours or less. The accompanying table, which shows the progressive appearance of the

different stages of development in a typical fly thus examined, supports the view we have expressed. Supplementary evidence is provided by the observation that daughter (short) crithidia were regularly present in the 'saliva' of all flies which later proved infective and capable of transmitting *T. brucei* to susceptible animals, whereas flies which failed to transmit the disease revealed daughter crithidia in the 'saliva' on very rare occasions or not at all. In some cases (Lloyd, 1930) it is likely that all flagellates in the proventriculus may be swept back to the gut by subsequent feeds of blood. Our conclusions do not affect the postulates of Robertson (1913*b*) in regard to the factors that influence infectivity. They differ, however, from her remarks and those of Lloyd and Johnson (1924) that the long proventricular trypanosomes pass into the salivary gland, settle down and attach themselves to develop into crithidia and ultimately to metacyclic trypanosomes. We agree with Brumpt (1922) in that the glands are invaded, not by the long slender forms, but by the short daughter crithidia which attach themselves without such further great changes as would be necessary in the longer trypanosomes and crithidia.

TABLE

Showing a periodical rise and fall in the relative abundance of proventricular forms and the progressive initial appearance of the different developmental forms of *T. brucei* in a fly whose saliva was examined daily. On the fourth and fifth days the fly failed to 'salivate'

Days from first appearance of trypanosomes	Proventricular forms	Post-proventricular tadpole forms	Daughter crithidia	Metacyclic trypanosomes (early forms)	Metacyclic trypanosomes (late forms)
3	×	—	—	—	—
6	×	×	—	—	—
7	×	×	—	—	—
8	×	×	—	—	—
9	×	×	×	×	—
10	×	×	—	×	×
11	×	×	×	×	×
12	×	×	×	×	×
13	×	×	×	×	×
15	×	×	×	×	×
17	×	—	×	×	×
18	—	—	—	×	×

It is interesting to note that Robertson (1913*b*) illustrates the post-proventricular tadpole-like flagellate for *T. gambiense* in *G. palpalis*, and comments on it as follows:

'The posterior position of the nucleus sometimes seen in these [slender] forms seems to be due to the tendency so often shown in degenerating trypanosomes for all the protoplasm and its contents to aggregate at the posterior end (Fig. 41). This condition does not seem to me to be really in any way comparable to the usual crithidial phases of trypanosome cycles, where the whole culture passes through a state in which the tropho-nucleus is definitely posterior to the kinetonucleus, and which is followed by a re-assumption of the trypanosome-condition proper (compare the life-cycles of *T. lewisi*, *T. raiae* . . . and the salivary gland phases of *T. gambiense*).'

• Fraser and Duke (1912, fig. 7) show a figure of an aflagellate form of *T. gambiense* from the proventriculus of *G. palpalis* suggestive of our daughter crithidia; and Taylor (1932) refers to rather rare short stumpy crithidia in the proventriculus of *G. tachinoides* infected with *T. gambiense*. Do they occur in the three polymorphic trypanosomes of

Africa, *T. gambiense* and *T. rhodesiense*, in addition to *T. brucei*? Are they also an essential part of the exogenous cycle in *T. congolense* and *T. vivax*? And do they feature in the development of *T. evansi* in tabanids? To us, it is evident from a perusal of the literature and from our own experience—often incidental to our experiments on transmission of trypanosomiasis to animals—that more detailed information is required, and obtainable, to complete our knowledge of the development of trypanosomes and of the factors that influence this development in tsetse-flies.

Finally, the investigations which we have carried out show that *T. brucei* develops more readily and produces daughter crithidia more frequently in *G. pallidipes* than in *G. austeni* reared together under a variety of conditions, including a temperature of 30° C. for pupae and flies, and fed on several occasions on the same reacting animals.

### SUMMARY

1. A detailed account, based on examination of flagellates in the 'salivary' exudate, is given of the development of *Trypanosoma brucei* from the proventricular to the infective metacyclic stages in *Glossina pallidipes* and *G. austeni*.

2. The sequence of development includes a post-proventricular phase of unequal fission or simple gemmation producing short aflagellate crithidia which invade the salivary glands and continue the exogenous cycle to the final metacyclic trypanosomes.

3. The fate of the proventricular trypanosomes and of the long slender crithidia is explained.

4. The rounded forms of earlier workers are excluded from the normal sequence of development of *T. brucei* in the fly.

5. *T. brucei* develops more readily and produces daughter crithidia more frequently in *G. pallidipes* than in *G. austeni*.

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# OBSERVATIONS ON THE USE OF SODIUM ANTIMONY GLUCONATE (SODIUM STIBOGLUCONATE) IN THE TREATMENT OF KALA-AZAR

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## INTRODUCTION

In 1942 we received a communication from Dr. C. M. Wenyon, F.R.S., stating that the Wellcome Chemical Research Laboratories had produced a compound, sodium antimony gluconate, which behaved in animals in the same way as the Bayer preparation solustibosan, and asking if we should be interested to have a supply of the compound for clinical trial in human leishmaniasis. We were very willing to accept this invitation, and the present communication is a report of our experiences with the drug.

Sodium antimony gluconate, or sodium stibogluconate, as it is now called by the makers, forms a colourless, stable, aqueous solution. All the cases reported in this paper were treated with a solution of the drug prepared by the manufacturers in England and supplied to us in sealed glass ampoules of 6 c.cm., sometimes after long delay in transport. The first batches of the drug sent to us contained in each cubic centimetre of the solution 75 mgm. of the compound, equivalent to 20 mgm. of pentavalent antimony. During the period October, 1943, to May, 1944, we treated 12 cases with this preparation, using conservative and orthodox schemes of dosage similar to those previously used with solustibosan. In a letter dated August 8th, 1944, Dr. Wenyon recommended the administration of two ampoules a day instead of one, and, quoting the work of Kikuth and Schmidt (1943) and of Lozano Morales (1943) in Spain, suggested that more than two ampoules a day could probably be given for 10 days. With the next 10 cases, treated during the remainder of 1944 and 1945, we considerably varied the schemes of dosage, sometimes giving up to four ampoules daily. In 1945 we received a supply of the drug in a concentrated solution, five times as strong as the original one, so that each cubic centimetre contained the equivalent of 100 mgm. of pentavalent antimony. This solution was likewise supplied in ampoules of 6 c.cm. Fourteen cases have been treated with the concentrated preparation.

## CLINICAL MATERIAL AND METHODS

Except for two patients who were treated in Khartoum, the cases reported in this paper were all kala-azar cases coming to Gedaref Civil Hospital for treatment. No selection of cases has been made, except in so far as the feasibility of following up the patient after discharge from hospital was considered likely or otherwise. This was necessary because the population of the Gedaref area includes many immigrants, mostly westerners, who move from place to place seeking casual employment, and such persons can never be traced after discharge from hospital. We believe that the cases here reported constitute

a fairly typical random series of cases of Sudan kala-azar. All degrees of clinical severity are represented. In all instances diagnosis was confirmed by finding leishmania in spleen smears or lymph-gland juice or both.

The general management of the cases during treatment was similar to that which we have used with other specific remedies in the past. All of them were admitted to hospital and treated as in-patients. The criteria of presumptive cure adopted by us, to which frequent reference will be made, have already been described in detail (Kirk and Sati, 1940*a*, 1940*b*).

In the cases under review the drug was invariably given intravenously. No other route has been tried, although the compound is said to be suitable for intramuscular use. Each injection was 6 c.cm. of either weak or concentrated solution, i.e., the contents of one ampoule as supplied by the makers. In some of the cases treated with the weak solution two, three, and even four ampoules were given daily, but always in divided doses throughout the day, each dose being 6 c.cm. or one ampoule. No dose less than 6 c.cm. of either the weak or the concentrated solution was given, even to children.

TABLE I  
First series of cases on the weaker solution (20 mgm. Sb per c.cm.)

Case no.	Sex	Age (years)	Weight (kgm.)	No. of injections	Total Sb (gm.)	Rash	Days in hospital	Days of treatment	Follow-up (months)	Complications
1	Male	37	57	20	2.4	+	109	41	24	M.T. malaria
2	"	27	63	30	3.6	+	86	45	24	M.T. malaria, pneumonia
3	"	4	13	20	2.4	+	87	44	24	M.T. malaria
4	"	45	48	20	2.4	—	71	27	23	—
5	"	38	42	40	4.8	+	81	40	22	—
6	"	7	22	40	4.8	+	99	61	16	—
7	"	7	15	40	4.8	+	90	61	20	M.T. malaria, albuminuria
8	"	28	—	30	3.6	—	111	—	7	—
<b>DEATHS</b>										
9	Male	25	—	7	0.84	—	—	—	—	M.T. malaria, albuminuria, diarrhoea
10	"	30	—	7	0.84	—	—	—	—	Pneumonia
11	"	22	45.5	24	2.88	—	—	—	—	M.T. malaria, albuminuria, pneumonia
12	"	50	—	40	4.8	—	—	—	—	—

Average total dose required for cure = 3.75 gm. pentavalent antimony.

#### FIRST SERIES OF CASES TREATED WITH THE WEAK SOLUTION (20 MGM. PER C.CM.)

In this series treatment was given in courses of 10 daily injections of 6 c.cm. The courses were separated by observation-periods which varied from a few days to a month, according to the progress of the patient. No reduction of the dose was made for children, some of whom required no less than four courses of full adult dosage to effect cure. The results in this series are shown in Table I. There were four deaths and eight cures, most of which have now been followed up for a considerable period without showing any



relapse. Cases which developed a post-kala-azar skin condition during or after treatment are indicated in column 7. The sign + indicates a well-marked and conspicuous eruption (the sign ± in the later tables indicates an inconspicuous skin eruption). Post-kala-azar dermal manifestations were more frequent and more conspicuous in this series than in the two later series, in which a more intensive form of treatment was adopted.

The deaths in this series fall into two groups. No. 10 was an Abyssinian admitted in a very debilitated, almost moribund, condition, with severe epistaxis, pneumonia and muttering delirium. No. 9 had severe diarrhoea, jaundice, heavy albuminuria and a puffy face on admission. Both died after seven injections. These two cases form the first group, and were simply too ill on admission for any chance of recovery.

The other two fatal cases had a fair amount of treatment. No. 11 died possibly as the result of his severe complications. No. 12 failed to respond to treatment, and died of kala-azar without any obvious complications. Post-mortem examinations were obtained in both cases and showed the principal visceral tissues still full of leishmania. The conclusion is therefore that the drug failed to control the infection in these cases, in the doses used.

TABLE II  
Later cases treated with the weaker solution (20 mgm. Sb per c.cm.)

Case no.	Sex	Age (years)	Weight (kgm.)	No. of injections	Total Sb (gm.)	Rash	Days in hospital	Days of treatment	Follow-up (months)	Complications
1	Male	25	48	40	4.8	+	88	27	12	Diarrhoea, M.T. malaria
2	"	38	47	30	3.6	+	107	34	8	M.T. malaria, Kahn ++, severe epistaxis
4	"	26	47	30	3.6	—	103	44	—	Relapsing fever
5	"	35	45	30	3.6	—	91	50	—	—
6	"	25	55	40	4.8	—	93	80	6	—
7	"	22	51	40	4.8	+	119	78	5	—
8	Female	30	39	40	4.8	—	43	18	5	—
9	Male	20	45	20	2.4	±	106	5	1	—
10	"	21	40.5	20	2.4	±	87	5	1	—
DEATH 3	Male	40	51	30	3.6	—	Died in hospital			Pneumonia

Average total dose required for cure = 3.87 gm. pentavalent antimony.

#### LATER CASES TREATED WITH THE WEAK SOLUTION

The second series of cases treated with the weaker solution (20 mgm. pentavalent antimony per c.cm.) consists of 10 cases, with one death and nine apparent recoveries. The details are summarized in Table II. In this series we considerably varied the schemes of dosage, giving two, three, and even four ampoules of the solution per day. Some notes on the schemes of dosage used are given below.

*Case No. 1* had 40 injections, in two courses of 10 days (two injections daily) separated by an interval of seven days.

*Case No. 2* had a 10-day course of two injections daily, followed, after 12 days' rest, by a 10-day course of one injection daily.

*Case No. 4* had a 10-day course of two injections daily, followed, after 24 days, by a 10-day course of one injection daily.

*Case No. 5* had a 20-day course of one injection daily, followed, after 19 days, by a 10-day course of one injection daily.

*Case No. 6* was started with a 10-day course of one injection daily, then had 12 days' rest. Thereafter he had another 10-day course of one injection daily, followed, after seven days' rest, by a third 10-day course of one injection daily. This was followed by a 22-days' observation-period, during which it was evident that the condition had not yet subsided, so a fourth 10-day course of one injection daily was given.

*Case No. 7* had a 10-day course of one injection daily, followed, after 10 days' rest, by another 10-day course of one injection daily. The fever persisted during the following eight days, so a third 10-day course of one injection daily was given. This brought down the fever, but after eight days it rose again with some re-enlargement of the spleen. Observation during the following 10 days indicated that this was an exacerbation of the leishmania infection and not the result of some complication, so a fourth 10-day course of one injection daily was given.

*Cases No. 8, 9 and 10.* It was evident by this time that better results followed the more intensive forms of treatment; so in the next three cases the drug was given four times daily, at four-hourly intervals, in five-day courses. Case no. 8 had two such courses separated by a week's rest-period, and reacted very satisfactorily. Nos. 9 and 10 had only one such course. This appears to have effected cure in both cases, in as short a period as five days. This is a surprising result in Sudan kala-azar, and hence the patients were retained in hospital for very long observation-periods (without further treatment) before we felt justified in regarding them as provisional cures. During this period the tests of presumptive cure described by us (1940) were, however, satisfied. The follow-up in both cases is as yet short, and it remains to be seen what their future progress will be.

*Note on the Fatal Case.* The patient was admitted on March 5th, 1945, with the usual fever and palpable spleen and liver. Right ear discharging pus, left ear-drum red and inflamed. Blood: red cells, 3,200,000; white cells, 4,000; differential count, neutrophils 68 per cent., lymphocytes 28 per cent., large mononuclears 3 per cent., eosinophils 1 per cent. Urine and stools clear. No malaria or relapsing fever in blood films. Widal's reaction was negative for typhoid, paratyphoid and Malta fever. Spleen puncture was negative twice; gland puncture was negative four times, but positive the fifth time. The patient was given a 10-day course of three injections daily. After that he unfortunately developed double pneumonia, which proved fatal nine days after the completion of the course of injections.

### CASES TREATED WITH THE CONCENTRATED SOLUTION

Fourteen cases were treated with the concentrated solution (100 mgm. of pentavalent antimony per c.cm.), with one death and 13 apparent recoveries. The results are summarized in Table III.

In the first case in this series treatment was started with a course of one injection (6 c.cm.) daily for four days. This brought down the fever rapidly. For the next 24 days the patient was almost afebrile, apart from slight evening rises of temperature on three occasions, the highest of which was 100.6° F.; during this period gland and spleen punctures were both negative for leishmania on three occasions. On the 25th day,

however, the fever rose again sharply, and assumed a swinging type. The patient's general condition deteriorated. Spleen and liver re-enlarged, and leishmania were readily found in spleen and gland punctures—an obvious relapse. He was given another course of one injection daily for four days. The first two injections had no effect on the fever, but by the time the fourth had been given the temperature came down again and remained down during the following 26 days, when the other tests of cure were carried out satisfactorily. The patient was then discharged as an apparent cure, after having received only eight injections.

In subsequent cases treatment was started with a course of one injection daily for four days; then, after a week's rest, tests of cure were carried out. In no case were the criteria of cure recommended by Kirk and Sati (1940*a*, 1940*b*) satisfied at this early stage; so, in view of experience with the first case, two more injections were given on consecutive

TABLE III  
Cases treated with the concentrated solution (100 mgm. Sb per c.cm.)

Case no.	Sex	Age (years)	Weight (kgm.)	No. of injections	Total Sb (gm.)	Rash	Days in hospital	Days of treatment	Follow-up (months)	Complications
1	Male	29	51	8	4.8	—	66	33	11	Kahn + + +
2	"	30	57	6	3.6	±	68	20	10	—
3	"	25	52	6	3.6	+	41	23	9	Amoebic hepatitis
4	"	32	43	6	3.6	—	56	20	10	M.T. malaria, jaundice
5	"	27	50	6	3.6	—	32	19	7	Kahn + +
7	"	55	48	6	3.6	—	57	15	9	M.T. malaria
8*	"	22	55.5	8	4.8	±	126	43	6	M.T. malaria, amoebic hepatitis
9*	Female	40	45	6	3.6	—	66	15	7	Severe diarrhoea
10	Male	26	53.5	10	6.0	—	75	62	5	Pneumonia
11	"	5	14.5	6	3.6	—	58	15	4	—
12	"	22	45	9	5.4	—	138	84	2	M.T. malaria, pleurisy
13	"	29	53	6	3.6	±	53	6	4	M.T. malaria
14	"	30	50	6	3.6	—	57	6	4	—
DEATH 6	Female	4	13.5	4	2.4	—	Died in hospital			M.T. malaria, cancrum oris

\* Previously treated unsuccessfully with penicillin.

Average total dose required for cure = 4.1 gm. pentavalent antimony.

days after a lapse of 14 days from the end of the first course. This routine appears to have been satisfactory. After a suitable rest-period, tests of cure were again carried out and were repeated time and again until the evidence pointed to a satisfactory provisional cure.

This was the procedure followed in the cases shown in Table III as having had six injections, with the exception of the last two (nos. 13 and 14). It was decided in these two cases to try the effect of cutting out the rest-period of 14 days which separated the first four injections from the last two. The whole treatment was given in six consecutive days, with successful results as regards immediate cure. The period of follow-up is still short in both cases, but so far there has been no indication of relapse.

Three cases in which the period of treatment was prolonged require some comment.

Case No. 8 had previously been given penicillin, with no beneficial effect. He was given four daily injections of sodium stibogluconate, followed, after an interval of 10

days—during which he was afebrile—by another two injections, in the usual way. He remained afebrile for the following 15 days, then developed fever, headache, diarrhoea, and signs of acute hepatitis. *E. histolytica* was found in the stool. The fever and other symptoms subsided under emetine treatment, but at the end of this it was considered safer to give him another two injections of the stibogluconate, in case the leishmania infection had played a part in the relapse. This accounts for the prolongation of the period of treatment.

*Case No. 10* was started with the usual four daily injections. This had no effect whatever on the fever, so after a week's interval he was given another four, after which the fever settled. He continued afebrile during the next month, with four negative spleen and gland punctures to his credit in this period, and might have been discharged as cured had not the fever then recurred, with some re-enlargement of the spleen. Blood films were negative for malaria, and the fever did not react to mepacrine; so a further two injections of sodium stibogluconate were given on the 61st and 62nd days from the beginning of treatment. This brought down the fever rapidly and permanently.

*Case No. 12.* Treatment was started in this case with six injections on consecutive days. The fever did not immediately subside, and physical examination revealed pulmonary congestion with associated pleurisy at the right base. After a few days' treatment with M. and B. 693 the fever and physical signs disappeared. About a month later fever recurred and malignant tertian malaria was found in the blood. The fever did not react to mepacrine, but ran an irregular course for the following month. A spleen puncture was then found to contain leishmania, but in the meantime the fever appeared to have subsided under quinine treatment. Three further daily injections of sodium stibogluconate were given on the 82nd, 83rd and 84th days of treatment, and during the subsequent observation-period satisfactory tests of presumptive cure were obtained.

*Note on the Fatal Case.* The patient was a little girl, aged four, who was extremely ill and anaemic on admission, and, in addition, had malignant tertian malaria. She had been ill for five months, and for the last four weeks of illness had been bed-ridden. Blood: red cells, 1,200,000; white cells, 1,600; haemoglobin, 26 per cent.; differential count, neutrophils 52 per cent., lymphocytes 36 per cent., large mononuclears 12 per cent., eosinophils 0 per cent. She was given four daily injections (full adult doses), but this had no effect on the fever. Two days later cancrum oris and purulent aural discharge made their appearance, and the patient died in the next week. Treatment was given for these conditions, as well as for the malaria and the kala-azar. But the patient was a late case, practically moribund on admission, and it was impossible to save her.

### TOXICITY

Toxic effects following the injections were negligible. In one patient treated with the weaker solution (20 mgm. Sb per c.cm.) the second, third and fourth injections were associated with rigors. The only toxic symptoms recorded with the concentrated solution (100 mgm. Sb per c.cm.) were, in one case, transient short cough and a feeling of tightness behind the sternum on administration of the first three injections. The same patient vomited once after the injection. These symptoms passed off in a few seconds after the cessation of the administration of the dose. No other toxic effects were observed.

It may be noted that in three of the six fatal cases death was associated with pneumonia. It has always been said that antimony, especially the trivalent form, is liable to

produce pulmonary complications, including pneumonia. In a small series like the present one, however, it is very doubtful if this complication can be attributed to the drug, since it is known that kala-azar patients are particularly liable to pneumonia, even without antimony.

### COMMENTS

The difficulties encountered in the antimony treatment of Sudan kala-azar have been emphasized by various workers (Archibald, 1923; Henderson, 1937; Horgan and Kirk, 1940; Stephenson, 1940; Kirk and Sati, 1940). Practically all are in agreement that treatment is long and expensive, the results disappointing, and the mortality-rate high. The best results are those reported by Sati (1942), in which the immediate cure-rate was 77 per cent., with prolonged hospital treatment and much larger total doses of pentavalent antimony than those found effective by Indian workers in Indian kala-azar.

As regards the present series, it seems to us that the results in the first group are similar to those to which we have been accustomed in the past, but those in the second and third groups—especially the latter—are different. The mortality-rate is lower, but that is not of great significance owing to the small number of cases involved. A more significant feature is the reduction in the length of treatment necessary for cure with the more intensive schemes of dosage made possible by the low toxicity of the new compound.

When one experiments with a new method of treatment in a disease like kala-azar, the tendency is to play for safety and to keep the patients in hospital for long periods of observation, until one feels reasonably justified in regarding them as provisional cures. This factor has undoubtedly increased the period of hospital treatment in some of the present cases. In others, prolonged hospital treatment was necessary for the complications rather than for the kala-azar. In two cases unsuccessful attempts were made to treat the disease with penicillin (Horgan and Sati, 1945) before sodium stibogluconate was used. It seems hardly justified to include such considerations when assessing the period of hospital treatment required for cure, but allowance has not been made for such factors in previous reports from the Sudan. In the tables summarizing the results of treatment in the present series we have therefore shown two columns referring to length of hospital treatment: one gives the total period in hospital from the date of admission to the date of discharge; the other shows the time between the first and last injection of specific treatment, including in-between rest-periods and periods of observation, but not the time spent before the start of treatment in establishing the diagnosis, nor the final observation-period after the completion of treatment. From the figures it will be seen that some of the cases on the more intensive forms of treatment have apparently been cured by courses of treatment lasting for less than three weeks—in some cases for no more than five or six days.

This is a very remarkable result in Sudan kala-azar. There is reason to hope that further work along the lines here indicated may, by reducing the long period of hospitalization hitherto found necessary for cure, solve one of the main difficulties in the treatment of Sudan kala-azar. It may even be possible in the future to treat selected cases in the out-patient department—a course which would not at present be considered with any other drug by a physician with experience of Sudan kala-azar. Owing to its stability in aqueous solution, and to the ease and convenience of its administration, sodium stibogluconate seems to us a suitable preparation for issue to rural hospitals and dispensaries.

Before the recent war, results obtained in the Sudan with solustibosan (Bayer) were no better than those obtained with other antimony preparations. The drug was found less toxic but less effective than neostibosan in the doses recommended by the manufacturers. There seems little doubt that the greater success in the present series has been due entirely to the different system of dosage employed. In the first group the dosage and results are similar to those previously experienced with solustibosan. With the most intensive systems of dosage used in groups II and III the parasites appear to have been eliminated in some cases in five or six days. The time-concentration factor in dosage is apparently of more importance than the total quantity of drug used in treatment.

Finally, we suspect that the results here reported are not without relevance to the so-called 'antimony-resistant' cases commonly encountered in Sudan and Mediterranean kala-azar. It has been noted in the Sudan (cf. Sati, 1942) that cases in which the physician gives small initial doses, gradually working up to the full dose, are always more difficult to cure than those which are given maximum doses from the outset. Should the more intensive treatment here described ultimately win the approval of our colleagues in the Sudan we hope that another bugbear in the treatment of kala-azar—the antimony-resistant case—will be less frequently encountered.

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# ATTEMPTS TO INDUCE BLACKWATER FEVER EXPERIMENTALLY

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Many theories have been advanced to explain the acute haemolysis of blackwater fever by the presence of autohaemolysins in the blood. The essential similarity between the lesions produced by the injection of either an antihaemolytic serum or an incompatible blood transfusion and by paroxysmal haemoglobinuria in man, in all of which there is known to be an antigen-antibody reaction, has suggested the possibility that a similar mechanism may be responsible for the acute haemolysis of blackwater fever.

There is also evidence to show that it is during the process of immunization to particular strains of malarial parasites, especially of *Plasmodium falciparum*, that the liability to develop blackwater fever occurs.

Europeans, as shown by British troops in West Africa during the recent war, rarely became liable to blackwater fever till they had been resident in the tropics for at least nine months. Adult Africans develop blackwater fever very infrequently. The majority of cases in Africans are in children of from 6 to 12 years old, while of those seen in adults a considerable proportion is in Africans who have adopted antimalarial precautions by taking suppressive drugs and by using mosquito-nets. Blackwater fever is thus most likely to occur in Africans who have not yet acquired immunity or are in process of losing it. It is known that immunity to malignant tertian malaria is lost comparatively quickly. African students who have resided outside West Africa for two years or more find that when they return they are liable to suffer from malaria as severely as they did in early childhood, unless they take antimalarial suppressive drugs.

If it were possible to reduce the immunity of a given body of adult Africans to malaria while they still remained in West Africa it might be expected that they too would show an increased incidence of blackwater fever. Now, during the war African soldiers have had considerable opportunities for reducing the frequency with which they have been bitten by infected *Anopheles*; they have lived for the most part in well-sprayed quarters and in areas which have been carefully sanitated and controlled for mosquito-breeding, and, although they have not at any time received antimalarial suppressives, many have learnt to use and appreciate mosquito-nets.

The yearly incidence of blackwater fever among African troops in West Africa is shown in the following table.

It will be seen that the case-incidence has gradually increased and was much higher in 1944 and 1945 than in the three earlier years. Two factors may here be relevant: antimalarial drainage schemes initiated in 1941 and 1942 did not begin to exert their effect till well on into 1943; as the control-measures proved more efficient their effects were even greater in 1944 and 1945. The length of service of the West African soldier

TABLE  
Incidence of blackwater fever in African troops

Year	No. of cases	Incidence per 10,000 strength
1941	1	0·23
1942	6	0·68
1943	13	1·30
1944	36	5·49
1945	66	12·77

was greatest in 1944 and 1945, since recruiting was most active during 1941, 1942 and 1943. In the last two years there was thus an African population which had been living in well-controlled areas for a considerable period, during which the chances of reinfection were obviously much less than they would have been had the same men been living in their own villages, although the chances of reinfection were not entirely absent.

In view of the dramatic increase in the number of cases of haemoglobinuria among West African troops during 1944 and 1945, it is pertinent to ask whether any similar increases were observed in the civilian African population over the same period. Figures for African cases of blackwater fever, which will be published in full elsewhere, have kindly been supplied by the Directors of Medical Services in the four British African Colonies and by the Médecins en Chef of French Togoland and the Belgian Congo. There is no apparent increase in the incidence of blackwater fever in any of these areas, except in 1936 in the Belgian Congo, when, instead of an average of 8–15 cases a year, the number suddenly rose to 121. This large increase, Major-General van Hoof informs us, was due to the arrival at the mines in the province of Costermanville of a large number of workers from the north-eastern part of the Belgian Congo, Ruanda-Urundi, where there is little malaria; the region of the mines, on the other hand, is a highly malarious area. Of the 121 cases of blackwater fever in 1936 no fewer than 111 occurred in these immigrant mine-workers. This is, therefore, an instance of a high blackwater fever incidence among a non-immune African population.

In considering other possibilities which might have weighted the scales in favour of a large increase in haemoglobinuria in African soldiers as compared with African civilians during 1944 and 1945, the question of the supply of sulphonamide drugs was investigated. Individual case-histories did not suggest that sulphonamides were related in any way to the onset of the haemoglobinuria, except in one instance; sulphonamides are now used as freely in civilian hospitals in Africa as they are in military establishments, and, though there has always been a 'black market' in the sulphonamides in West Africa, yet in 1941 and 1942 this market was no less extensive and was certainly less well controlled than in 1944 and 1945.

There is, however, one other group which is strictly comparable to West African troops in West Africa, and that is the West African troops who were sent to India and Burma from the middle of 1943 onwards. Full figures for South-East Asia Command are not available, but among West African troops in India with an average monthly strength of from 5,986 to 4,590 there were from January, 1944, to the end of 1945 only two cases of haemoglobinuria. No cases occurred in West Africans in S.E.A.C. in 1945, and no West Africans were invalided home from S.E.A.C. with blackwater fever at any



time, so that there cannot have been any large number of cases in the two West African divisions while actually in face of the enemy. Now, in India the factors which are thought to precipitate attacks of blackwater, such as chills, fatigue and emotion, were just as, if not more, likely to involve Africans than in West Africa; the venereal disease rate, and thus the use of sulphonamides, was not significantly lower in India than it was in Africa. There was, however, one striking difference: West African troops who had gone to India were not being reinfected with West African strains of *Plasmodium falciparum* as were those at home in West Africa.

As a working hypothesis, therefore, it was suggested that blackwater fever is liable to occur in those populations where there is a low degree of immunity to a particular strain of malignant tertian malaria parasite when they receive an additional dose of the same malaria parasite, the low degree of immunity being due to the fact either that complete immunity has not been acquired or that it has been lost.

### EXPERIMENTAL OBSERVATIONS

The above theory appeared to be susceptible of experimental investigation. The experiments were carried out on Africans. In a particular hospital all patients, after discharge from the wards, were sent to a convalescent wing where they received rehabilitation treatment.

#### *Experiment 1*

Three Africans received 5 c.cm. of normal blood intramuscularly into the right buttock. The Africans were convalescent from blackwater fever, having ceased to haemolyze from 28 to 45 days before the injection was given. Over an observation-period of four weeks there was no sign of illness and no evidence of haemoglobinaemia or haemoglobinuria.

#### *Experiment 2*

Six Africans similarly convalescent from blackwater fever for from four to six weeks, and four controls who had recovered from surgical complaints, were each given 5 c.cm. of blood into the right buttock, the blood being removed from a patient during an attack of malignant tertian malaria. (An examination of thick films of the blood with an oil-immersion lens showed approximately 10 parasites in 50 fields.)

The four controls showed no reactions during an observational period of a month.

Of the six convalescent blackwater fever patients three developed typical attacks of blackwater fever two, three and nine days after the injection; a fourth developed a febrile attack with enlarged spleen and malaria parasites in the blood on the 13th day after injection; the other two remained in good health.

#### *Experiment 3*

The three patients who had had a second attack of blackwater fever, and the one who had had an attack of malaria, were again given, as before, 5 c.cm. of blood containing malarial parasites 5-6 weeks after complete recovery from their attacks. Three normal controls were also given the same amount of blood intramuscularly. All Africans remained without reaction over an observation-period of one month.

## DISCUSSION

It will be seen that there is a similarity in these experiments to those of Fairley and Murgatroyd (1940), where in a woman convalescing from blackwater fever it was possible by administering quinine to induce a recurrence of haemolysis until a course of mepacrine was given, the capacity of quinine to produce blackwater fever appearing to be directly or indirectly related to persisting malarial infection. While it may have been pure chance that three of the six convalescent Africans inoculated with parasites duly developed a relapse of blackwater within 10 days of their infection, it is of interest that during this same period no other case of blackwater fever occurred in the whole Command with an African strength of 52,731.

The possibility that blackwater fever and other acute haemolytic anaemias are due to a process of sensitization has recently been put forward by Gear (1946). It is suggested by him that an autoantigen is produced by the action of the malaria parasite, or the malaria parasite plus an antimalarial drug, on the red blood-cell. This autoantigen gives rise to an autoantibody haemolysin which, reacting with red cells in the presence of complement, causes haemolysis.

The data here collected on the incidence of blackwater fever in various groups in West Africa, together with some experiments carried out in West Africa in 1945, by no means contradict this theory.

Our findings may be briefly summarized as follows. In European troops in West Africa blackwater fever rarely occurred within the first nine months of residence, the period presumably necessary for sensitization. Among African troops the incidence of blackwater fever was low in 1941 and 1942, but gradually rose during 1943, 1944 and 1945. African civilians did not show any similar rise in the incidence of blackwater fever. The only difference between civilians and soldiers was that the latter lived in areas where the chances of being bitten with infected anophelines were much reduced. African soldiers sent to India were also removed from the chance of being reinfected with West African strains of malignant tertian malaria, but they did not show a similar rise in the incidence of blackwater fever.

If in order to develop one attack of blackwater fever it is necessary to be sensitized to the malaria parasite, then it should be possible to induce a second attack in those who are still sensitized by injection of a fresh dose of antigen, just as a new attack of asthma may be induced by exposure to the correct allergen. A short series of experiments suggests that the induction of a further attack of blackwater fever is, in fact, possible by this means.

## SUMMARY

Evidence is brought forward in support of the view that sensitization is the cause of the acute haemolysis of blackwater fever.

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# THE MODIFICATION OF *TRYPANOSOMA RHODESIENSE* ON PROLONGED SYRINGE PASSAGE

BY

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Corson (1936) isolated a strain of *Trypanosoma rhodesiense* on October 21st, 1934, by feeding a box of clean laboratory-bred *Glossina morsitans* on an untreated case of Rhodesian sleeping sickness. One line of this strain has since been maintained in sheep, all transmissions being by cyclically infected *G. morsitans*.

From the 13th sheep host rats were infected (i.e., about October, 1936), and rats of either the 16th or the 18th rat passage were sent to the Wellcome Bureau of Scientific Research, arriving in London on March 17th, 1937. Since then this line has been maintained by syringe passage through mice, the animals now having an incubation-period of 2-3 days, and dying in 3-9 days.

Dr. C. A. Hoare, of the Wellcome Laboratories of Tropical Medicine, very kindly sent us some slides of this line, made on January 18th, 1946, after it had been passaged through 879 mice in London. We are indebted to him for the particulars of the transmission, and for informing us that this line was transmissible by tsetse (*G. morsitans*) some time in 1937, but that no further transmission details were available.

The morphology of the Tinde cyclically transmitted strain has already been described (Fairbairn and Culwick, 1946). It consists of three forms, a short, an intermediate and a long one, each form occurring as a positively and a negatively charged electrical variant, giving six types in all. The length-distribution curve for each type does not depart significantly from normality. The mean lengths of the *positively* charged variants in rats infected from sheep by fly-bite were:

Short form	...	...	...	19.30 $\mu$	$\pm$ 0.17 $\mu$
Intermediate form	...	...	...	23.00 $\mu$	$\pm$ 0.16 $\mu$
Long form	...	...	...	28.63 $\mu$	$\pm$ 0.19 $\mu$

An examination of the syringe-passaged substrain (in mice) from London was carried out. Both positively and negatively charged trypanosomes were present, the former greatly outnumbering the latter on the slides sent to us. Measurements of 300 positively charged trypanosomes were made from different parts of different slides, the fields being selected at random and all positively charged forms being drawn. The results are given in the following table.

The values of  $g_1$  and  $g_2$  show that the length-distribution curve does not depart significantly from normality, and we must therefore presume that the trypanosomes measured were all of one form. Fifty per cent. of the trypanosomes were in various stages of binary fission, and we have already shown in the cyclically transmitted strain that it is

TABLE

The mean length (in  $\mu$ ) of the positively charged trypanosomes of *T. rhodesiense* (syringe-passaged London substrain) and the statistical analysis of the data

Mean ... ..	23.29 $\mu$	Standard error $g_1$ ...	$\pm$ 0.141
Standard error ...	$\pm$ 0.12 $\mu$	$g_2$ ... ..	-- 0.294
$g_1$ ... ..	-- 0.068	Standard error $g_2$ ...	$\pm$ 0.280

only the long heterozygous form which divides in any numbers in the blood, and is possibly the only one which divides at all (Fairbairn and Culwick, 1946). It was therefore concluded that *all* the trypanosomes present in the syringe-passaged strain were the long heterozygous form of *T. rhodesiense*.

Not only has prolonged syringe passage produced a monomorphic strain consisting of only the long form, but the mean length of this form has been reduced to 23.29 $\mu$ , as opposed to a length of 28.63 $\mu$  for the long form in the cyclically transmitted strain, a reduction of over 5 $\mu$  in length.

## DISCUSSION

Murgatroyd and Yorke (1937) maintained a strain of *T. brucei* by various methods in guinea-pigs and mice.

Strain 2 was inoculated into mice in April, 1932, and was maintained in mice by syringe passage. By October, 1936, they found that the changes in the trypanosomes were very pronounced: the morphology became modified, the strain becoming typically monomorphic and exhibiting only slender forms with free flagella; 'the pathogenicity for mice greatly increased, and for guinea-pigs greatly decreased; the sensitiveness of the trypanosome to arsenicals was greatly enhanced; and the parasite became non-transmissible by *G. morsitans*.'

When the strain was first started, all guinea-pigs inoculated became infected, and the average length of life was 80 days. Between December, 1935, and May, 1936, the strain was again inoculated into guinea-pigs, but only two of the seven were infected. These two animals (no. 326 and another) lived for 144 and 130 days respectively, and it was found that 'the parasite had regained its pristine morphological characters and had become once more typically polymorphic with posterior-nuclear forms.'

Fairbairn and Culwick (1946) have shown that the stout and intermediate forms of *T. rhodesiense* are produced by syngamy which is greatly stimulated by a change of environment, and therefore the reappearance of polymorphism in these guinea-pigs seems to show that the strain was still capable of syngamy in certain circumstances, and, so far as at least some characters were concerned, had not been modified irreversibly. In May, 1936, a transmission from guinea-pig 326 (infected in January, 1936) was attempted, but none of the 131 flies surviving was infected; and it is a matter for regret that further attempts at transmissions were not made. Unfortunately, too, the arsenic-resistance of this now polymorphic strain in these guinea-pigs does not appear to have been examined, to see whether it also had changed.

Their strain 3, on the other hand, started in September, 1932, was maintained in guinea-pigs partly by blood inoculation and partly by *G. morsitans*. It was transmissible

by *G. morsitans* between September, 1932, and March, 1935, but when tested between January and September, 1936, it was no longer transmissible.

Up to December, 1935, the susceptibility of the strain to halarsol had scarcely changed. In October, 1936, however, the authors noted that the strain, now no longer transmissible, had become very sensitive to halarsol. At this time (October, 1936) the strain had undergone no morphological changes and was still typically polymorphic and showed numerous posterior-nuclear forms. A branch of this strain was sent to London on January 29th, 1936, and, after it had been syringe-passaged through a further 912 mice, Dr. Hoare kindly sent us some thin blood films made on January 18th, 1946. An examination of them showed that the strain was now monomorphic, consisting solely of long forms with a mean length of  $24.46\mu \pm 0.13\mu$  ( $g_1 = 0.0034 \pm 0.122$ ,  $g_2 = -0.267 \pm 0.243$ ), while in a strain of *T. brucei* still transmissible by *Glossina*, which we had the opportunity of examining, the mean length of the long forms was  $26.17\mu \pm 0.14\mu$ , while short and intermediate forms were plentiful, their mean lengths being  $16.59\mu \pm 0.09\mu$  and  $20.99\mu \pm 0.09\mu$  respectively.

In 1931 three strains of *T. rhodesiense* were inoculated from man into guinea-pigs and sent to Liverpool. Comparing these three strains with their old laboratory strain of *T. rhodesiense*, which had been maintained by syringe passage in mice since its isolation from man in 1923, Murgatroyd and Yorke (1937) found that these three strains were definitely resistant to arsenicals, while the old laboratory strain was extremely sensitive; and they remarked: 'If we regard the three strains as typical of untreated *rhodesiense* infections of man in East Africa, these observations afford an adequate explanation why aromatic arsenicals fail in the therapy of human *rhodesiense* infections.' They concluded that the sensitiveness to arsenicals must have been gradually acquired during the strain's prolonged passage through mice in the laboratory; and they quote Browning and Gulbransen, who found that in a strain of *T. brucei* (the same strain as that mentioned above) the trypanosomes became more sensitive to therapeutic substances after prolonged syringe passage through mice.

Our work has shown the marked morphological difference between two strains of *T. rhodesiense* of identical origin, one transmitted cyclically through *G. morsitans* and the other by syringe passage. Not only had the two homozygous forms, the short and the intermediate, disappeared from the latter strain, but the mean length of the remaining long heterozygous forms had decreased by over  $5\mu$ , and, by analogy with the *T. brucei* mentioned above, it is exceedingly doubtful if the syringe-passaged strain would now be transmissible.

In view of the progressive increase in arsenic sensitivity of syringe-passaged strains, and even of an increase in sensitivity when a strain ceases to be transmissible by *Glossina* (see strain 3), it seems that drugs which can act on strains which have become, or are becoming, genetically 'fixed' by manipulation in the laboratory do not necessarily act in the same way on strains which have retained their genetic plasticity by constant syngamy stimulated by frequently alternating changes of environment between various mammalian hosts and the fly, to which all naturally occurring strains are exposed. Hence it is no wonder that new drugs, which cure a *T. rhodesiense* infection in animals in European laboratories, do not have the same effect on the trypanosome in man in East Africa.

It must therefore be stressed that, if chemotherapeutic research in trypanosomiasis is to be really effective, all new drugs should be tested on strains of *T. rhodesiense* which are polymorphic and which have been, and are being, transmitted by *Glossina*.

## SUMMARY

1. A strain of *Trypanosoma rhodesiense* was transmitted in parallel from 1936 (a) by cyclically infected *Glossina morsitans* through sheep, and (b) by syringe passages through mice.

2. In January, 1946, an examination of these two lines showed that while the cyclically transmitted strain consisted of three forms—a short, an intermediate and a long—the syringe-passaged strain was monomorphic, and consisted solely of the long form.

3. While the mean length of the positively charged long form, in rats, in the cyclically transmitted line was  $28.63\mu$ , that of the positively charged long form, in mice, of the syringe-passaged strain was  $23.29\mu$ , a decrease in length of over  $5\mu$ . In the case of the cyclically transmitted strain, it has been found that a change of host does not alter the length of the long forms significantly.

4. It is shown from the literature that a strain which is syringe-passaged through mice gradually increases in its sensitivity to arsenicals, and evidence is adduced that this is the result of genetic segregation and a loss of plasticity in the strain, produced by constant conditions in the laboratory and the absence of the environmental changes occurring in nature, of which the most important is passage through the insect vector.

5. It is urged that all trials of new drugs should be undertaken with strains of *T. rhodesiense* which are polymorphic and have been, and are being, transmitted by *Glossina*.

ACKNOWLEDGEMENTS.—We have to thank the Honourable the Director of Medical Services, Tanganyika Territory, for permission to publish this paper.

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# STUDIES ON SYNTHETIC ANTIMALARIAL DRUGS XVIII.—THE ABSORPTION, DISTRIBUTION AND EXCRETION OF PALUDRINE IN EXPERIMENTAL ANIMALS

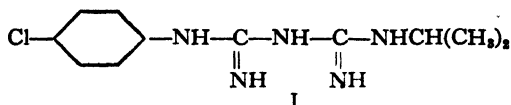
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## INTRODUCTION

Paludrine ( $N_1$ -*p*-chlorophenyl- $N_5$ -isopropylbiguanide, I) shows high therapeutic activity



against benign and malignant tertian malaria (Adams *et al.*, 1945; Maegraith *et al.*, 1945). Brief notes on its absorption, distribution and excretion have already been published (Spinks, Tottey and Maegraith, 1946; Spinks, 1946*a*). The experiments in the present paper were based on those already reported for 3349 (Spinks and Tottey, 1946*b*) and for 4430 (Spinks, 1946*b*). These had been devised to give information on certain properties that appeared to be characteristic of antimalarial drugs hitherto known; including strong affinity for tissue-cells, particularly leucocytes, and liver, lung, spleen and kidney; and a marked tendency towards excretion into the intestine. Paludrine was determined throughout by the hydrolytic method (Spinks and Tottey, 1945*b*, 1946*a*).

## EXPERIMENTAL SECTION

The techniques used have been fully described in previous publications and will be mentioned only briefly. Paludrine was administered as a 1 per cent. solution of the acetate.

TABLE I

Blood, plasma and tissue concentrations of Paludrine following the oral administration of 80 mgm. of base/kgm. in the rat

Time (hours)	Concentrations in mgm./l. or kgm.					
	Blood	Plasma	Liver	Lung	Spleen	Kidney
0.15	0.81					
0.30	1.20	0.29	20.3	5.96	4.99	8.32
0.50	1.40	0.48				
1.00			26.0	6.50	5.50	19.0
1.30	1.58	0.73	11.0	7.21	6.42	6.93
1.45	1.98	0.76				
2.30	1.26	0.59	20.3	6.81	12.8	4.95
3.30		0.47	6.0	3.62	5.01	5.75
5.00			5.07	8.03	3.70	4.93
7.00	0.70	0.24	6.75	4.75	3.70	4.80
16.00	0.67	0.16				
24.00			11.8	2.2	3.15	2.4

### *Absorption, Distribution and Excretion in the Rat*

Paludrine was administered by stomach-tube to groups of four rats, in doses of 80 mgm. of base/kgm. Concentrations (of base) found at intervals in pooled blood, plasma and tissues are recorded in Table I and diagram 1. Those found following a similar experiment in which Paludrine was administered intravenously in doses of 20 mgm. of base/kgm. are given in Table II.

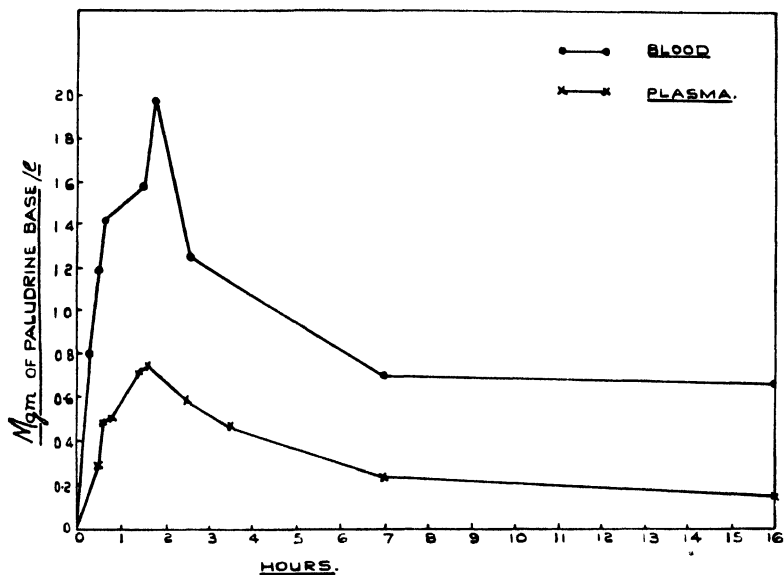


DIAGRAM 1. Blood and plasma concentrations of Paludrine in groups of four rats that received 80 mgm./kgm. orally.

TABLE II

Blood, plasma and tissue concentrations of Paludrine following the intravenous administration of 20 mgm. of base/kgm. in the rat

Time (hours)	Concentrations in mgm./l. or kgm.					
	Blood	Plasma	Liver	Lung	Spleen	Kidney
0-30	2.48	0.800	63.6	57.0	26.0	50.0
1-30	1.40	0.421	50.2	37.6	20.2	45.5
2-30	0.764	0.296	28.0	21.2	12.9	7.80
3-30	0.564	0.166	2.88	22.0	7.15	4.35
5-00	0.575	0.250	18.7	24.0	9.70	8.50
24-00	0.197	0.091	5.32	9.88	8.64	2.12

Paludrine is rapidly absorbed and gives somewhat higher concentrations in blood and plasma than 4430. It appears to be rather more persistent than the latter, but is much less firmly retained in the body than mepacrine. In distribution, as regards both blood/plasma and tissue/plasma concentration ratios it is very similar to 4430, standing with the latter in the series mepacrine, 3349, quinine, biguanides.

The excretion of Paludrine was examined following oral and intravenous adminis-



tration of the above doses to groups of six rats, kept in cages permitting separate collection of urine and faeces. The results are shown in diagram 2. Following oral administration 5.33 per cent. of the administered drug appeared in the urine and 12.9 per cent. in the faeces; following intravenous administration 33.48 per cent. appeared in the urine and 1.93 per cent. in the faeces. Again the similarity to 4430 is close, although the proportion of Paludrine excreted in the faeces is rather smaller than that of 4430. The low total recoveries suggest that Paludrine is largely metabolized in the rat. This possibility will be discussed below.

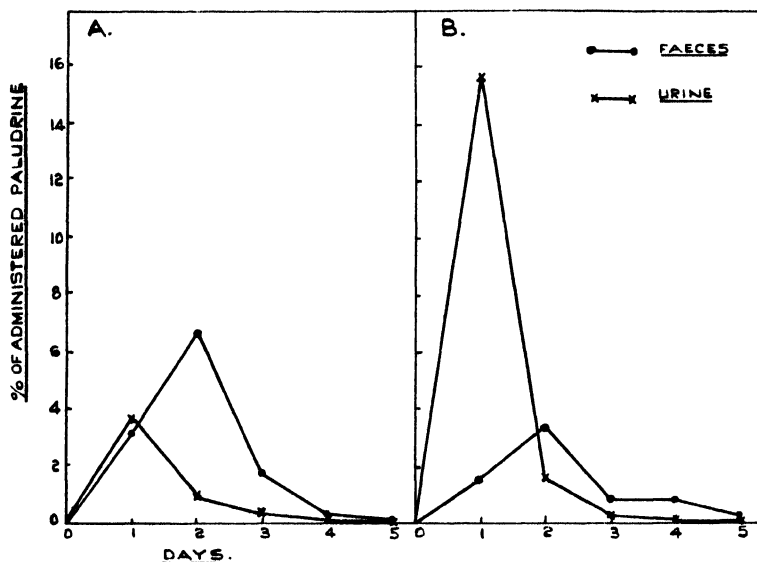


DIAGRAM 2. The excretion of Paludrine in groups of six rats following the administration of 80 mgm./kgm. orally (A) and 20 mgm./kgm. intravenously (B).

The excretion of Paludrine into the intestine was investigated by the analysis of intestinal contents following intravenous administration (Table III). Like 4430, Paludrine is excreted mainly into the small intestine, and only slowly into the large intestine.

TABLE III  
Paludrine in small and large intestines of groups of four rats receiving 20 mgm./kgm. intravenously

Time after dosing (hours)	Small intestine		Large intestine	
	Mgm.	% of dose	Mgm.	% of dose
0.30	0.464	3.00	0.035	0.23
1.30	0.467	3.16	0.083	0.60
2.30	0.448	2.61	0.116	0.67
3.30	0.447	3.75	0.332	2.77
5.00	0.845	4.22	0.725	3.62
24.00	0.442	3.25	0.636	4.54

The excretion in bile was examined by administering 20 mgm./kgm. intravenously to three rats having the bile-ducts cannulated (under chloralose anaesthesia). Concentrations found in pooled tissues and fluids after two hours were: blood 1.69 mgm./l.; liver 24.5, lung 9.4, spleen 11.1, kidney 32.4 mgm./kgm.; bile 1.33 mgm./l. (total 0.0598 mgm., 0.351 per cent. of dose); contents of small intestine (total) 0.159 mgm. (0.935 per cent. of dose); contents of large intestine 0.019 mgm. (0.112 per cent. of dose). Paludrine, like 4430 and 3349, is excreted into the intestine partly in the bile and partly by other routes. Since it can be demonstrated in the large intestine at short intervals after dosing (Table III) these other routes presumably include direct excretion into the lumen from the intestinal walls.

#### *Absorption and Distribution in the Mouse*

Concentrations of Paludrine found in the pooled blood and tissues of groups of six mice following the oral administration of 80 mgm. of base/kgm. are shown in Table IV. This dose was rather toxic in mice and several animals died, usually from two to seven hours after dosing. It was therefore necessary to use larger groups than six for later points. Although this procedure probably involved selection of the animals showing the lowest blood concentrations, it was adopted so that a rough comparison could be made with 4430 and 3349, which had been examined at the same dose (Spinks and Tottey, 1946*b*; Spinks, 1946*b*), as well as to give an indication of the lethal blood concentration in mice.

TABLE IV  
Concentrations of Paludrine in blood and tissues of mice following the oral administration of 80 mgm. of base/kgm.

Time (hours)	Concentrations in mgm. of base/l. or kgm.				
	Blood	Liver	Lung	Spleen	Kidney
0.30	2.55	90.0	10.4	10.0	17.0
1.00	5.38	92.5	34.0	14.9	13.5
1.30	9.05	137.0	35.0	26.5	49.0
2.30	5.10	80.0	30.5	42.5	108.0
3.30	2.90	86.0	22.0	13.2	73.0
4.30	3.15	57.0	31.5	15.1	75.4
7.00	2.35	34.0	26.0	10.4	52.0
24.00	1.15	22.2	12.0	8.93	24.1

Like 3349 and 4430 Paludrine gives much higher concentrations in the mouse than in the rat following the administration of the same dose per kilogram. It is similarly distributed in the two animals. The acute lethal blood concentration in the mouse is probably of the order of 10 mgm./l.

In the same experiment an attempt was made to determine Paludrine in the brain. An adequate technique had not been fully developed, but the likely error was in the direction of a high result due to the turbidity of the final extracts. Recoveries of Paludrine from brain were reasonably adequate (80–120 per cent.). Since in three of the eight analyses the concentration found was zero, and in all was less than 0.5 mgm./kgm., it is probable that Paludrine does not pass the blood-brain barrier. This tentative conclusion is supported by results in the rabbit, described below.

### Distribution and Excretion of Paludrine in the Rabbit

The distribution of Paludrine in the rabbit has been examined following the intravenous administration of 8 mgm. of base/kgm. Single rabbits were killed at the desired times by intravenous injection of 10 ml. of air. The results are given in Table V, and the blood concentration time-curves of rabbits 12 and 13 are shown in diagram 3.

TABLE V  
Concentrations of Paludrine in the rabbit following the intravenous administration of 8 mgm. of base/kgm.

Animal no. ...	3	1	5	6	10	9	11	13	12	8
Weight (kgm.) ...	2.0	2.37	1.8	2.3	2.2	2.35	2.9	2.2	2.8	2.7
Time ...	5 min.	10 min.	20 min.	30 min.	1 hr.	1½ hr.	2½ hr.	4 hr.	6 hr.	18 hr.
Blood (mgm./l.) ...	1.63	0.680	0.523	0.715	0.531	—	0.278	0.222	0.240	0.102
Plasma " ...	0.815	0.297	0.258	0.345	0.171	0.154	0.157	0.068	0.090	0.045
Bile " ...	—	0.79	—	—	6.31	—	—	—	4.61	1.51
Red cells (mgm./kgm.)	3.55	1.33	1.93	1.64	1.125	0.40	0.660	—	1.91	0.311
White cells "	(2.03)	(1.06)	—	—	—	(3.5)	—	—	—	(4.56)
Brain "	—	—	(0.91)	—	(0.56)	(0.33)	(0.90)	(0.34)	(0.58)	(0.46)
Liver "	32.1	37.5	21.7	26.7	15.9	5.47	6.53	2.17	2.18	0.701
Lung "	58.2	39.6	39.4	32.4	30.5	35.1	25.8	18.3	13.7	5.40
Spleen "	39.4	32.8	27.6	39.2	33.2	14.4	1.49	8.24	8.23	3.70
Kidney "	145.0	100.0	85.0	70.5	67.9	29.0	8.85	5.95	3.38	2.96
Muscle "	3.46	4.18	4.60	4.36	6.07	3.15	2.84	2.67	2.64	1.03
Fat (perirenal) "	4.41	2.06	2.46	2.15	1.51	1.19	0.31	0.79	1.08	0.128
Pancreas "	12.9	14.9	18.3	15.4	19.3	14.6	6.21	2.67	5.49	0.767
Intestine "	14.2	14.4	14.6	26.4	16.9	14.9	10.9	4.43	4.84	2.51
Heart "	—	—	—	21.8	25.2	14.9	9.88	4.97	2.66	1.24

Results shown in parentheses are to be accepted with reserve, those on white cells because it was found rather difficult to collect an adequate amount for analysis, those on brain because the analytical technique had not been fully developed. However, it is certain that the concentrations reached by Paludrine in white cells are much lower than those of mepacrine (Shannon *et al.*, 1944), and that the concentrations in brain are either very low or zero. In other respects the distribution of Paludrine is qualitatively very similar to that of mepacrine (Oldham and Kelsey, 1945) and the new I.G. quinoline derivative resochin (American chloroquine; Board for the Coordination of Malarial Studies, 1946), although quantitatively Paludrine differs in reaching much lower concentrations than these in all tissues. The approximate order of tissue concentration (in decreasing magnitude) was: kidney, lung, liver, spleen; heart, intestine, pancreas; muscle, fat; brain. The distribution did not differ markedly at five minutes as compared with later points, and it is clear that Paludrine diffuses rapidly from the blood into all tissues. The sharp initial fall in the blood concentration (see time-curves of diagram 3) also illustrates this rapid diffusion. The low concentration in fat is probably a reflexion of the fact that Paludrine, although freely soluble in lipoids as the base, is present at physiological pH almost entirely as the singly charged positive ion (J. C. Gage, private communication). The low or zero concentration in brain may perhaps be associated with the same property.

In order to provide a further type of compound carrying a dialkylaminoalkylamino

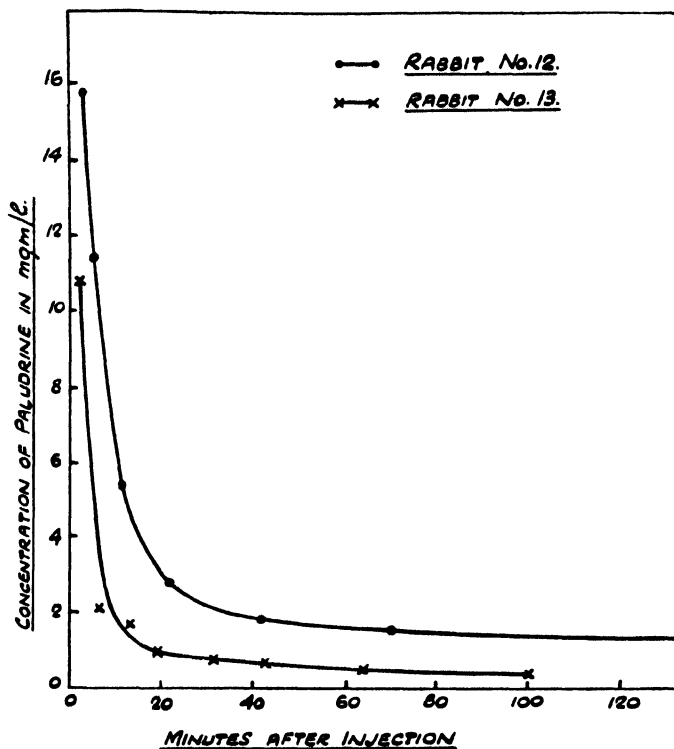
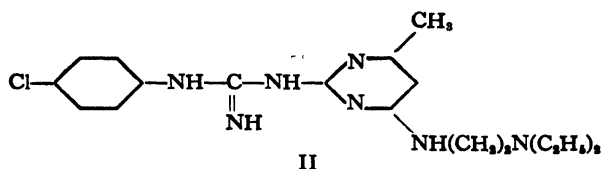


DIAGRAM 3. Blood concentration : time-curves of Paludrine in two rabbits that received 8 mgm./kgm. intravenously.

side-chain for comparison with Paludrine, the concentrations reached by 3349 (II) in fluids and tissues of the rabbit have been studied under exactly the same conditions,



using the method of determination already described (Spinks and Tottey, 1945a). The results are given in Table VI.

3349 is distributed qualitatively very similarly to mepacrine and resochin; quantitatively it stands between these and Paludrine. It was observed in the rat that the series of decreasing tissue/plasma concentration ratio was mepacrine, 3349, quinine, Paludrine; and this series appears to hold also for the rabbit. The lowest member of the series, Paludrine, differs markedly from freely diffusible drugs, e.g., the sulphonamides. The distribution of sulphaguanidine in rabbits following the intravenous administration of 50 mgm./kgm. is shown in Table VII. Sulphaguanidine was determined by the method of Rose and Bevan (1944).

TABLE VI  
Concentrations of 3349 in the rabbit following the intravenous  
administration of 8 mgm. of base/kgm.

Animal no. ...	15	16	18
Weight (kgm.) ...	3.1	2.8	2.4
Time ... ..	20 min.	2 hr.	18 hr.
Blood (mgm./l.) ...	1.73	0.371	0.066
Plasma " ...	0.677	0.125	0.022
Brain (mgm./kgm.)	1.47	0.414	0.300
Muscle "	4.36	2.66	—
Fat "	3.25	0.874	0.204
Intestine "	11.6	10.3	1.54
Pancreas "	27.2	26.7	3.51
Heart "	58.4	16.2	3.39
Spleen "	73.6	32.2	21.1
Liver "	19.7	17.7	9.79
Lung "	167.0	66.4	23.3
Kidney " "	146.0	46.3	36.6

The difference between sulphaguanidine and the antimalarial drugs is clear. The guanidine group does not markedly influence the distribution as compared to sulphanilamide, except as regards access to the central nervous system (cf. Fisher *et al.*, 1943). Otherwise, concentrations in tissue and plasma are of the same order.

Although sulphaguanidine and Paludrine both contain a guanidine residue, its structure at physiological pH cannot be the same in both, since sulphaguanidine is a very weak base (first  $pK$  about 2.75; Bell and Roblin, 1942) and is unionized, whereas Paludrine (first  $pK$  about 10.9; Gage, private communication) is present almost entirely as the singly charged positive ion. There is therefore no structural analogy between the two drugs.

TABLE VII  
Concentrations of sulphaguanidine in the rabbit following the  
intravenous administration of 50 mgm. of base/kgm.

Animal no. ...	19	20	21
Weight (kgm.) ...	2.2	2.0	2.6
Time ... ..	20 min.	1 hr.	2 hr.
Blood (mgm./100 m'l.)	8.1	0.92	0.55
Plasma " "	6.8	1.07	0.55
Bile " ...	—	1.82	—
Brain (mgm./100 gm.)	0.58	0.18	0.05
Muscle "	2.35	1.78	1.63
Fat "	1.18	0.12	0.03
Pancreas "	7.06	1.08	0.94
Intestine "	6.55	—	0.39
Heart "	6.71	1.41	1.20
Lung "	8.05	1.13	0.795
Liver "	2.29	1.96	1.24
Spleen "	7.18	0.73	0.935
Kidney "	19.8	3.72	1.86

The excretion of Paludrine in the rabbit is shown in diagram 4. Both oral and intravenous results are the mean of data from three animals. Following the oral administration of 80 mgm./kgm., 8.4 and 13.1 per cent. of the dose appeared in urine and faeces respectively. Following the intravenous administration of 8 mgm./kgm. the corresponding totals were 22.6 and 3.1 per cent. As in the rat, a considerable proportion of the drug must be metabolized. Some attempts have been made (with Dr. N. Senior) to investigate the route of this metabolism—so far with little success. There does not appear to be a rise in aromatic amine excretion following administration in the rabbit, and we have also been unable to detect any marked increase in glucuronide or ester sulphur excretion. These results will be reported elsewhere.

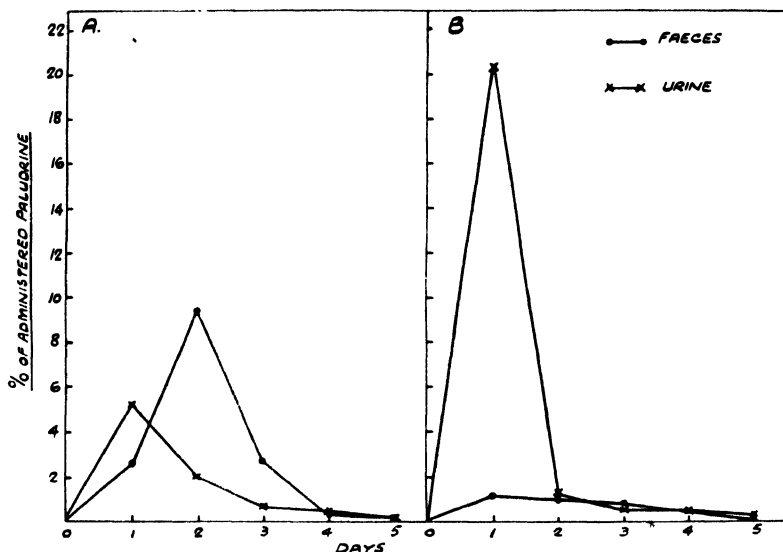


DIAGRAM 4. The excretion of Paludrine in rabbits following the administration of 80 mgm./kgm. orally (A) and 8 mgm./kgm. intravenously (B). Each is a mean of results obtained in three animals.

## DISCUSSION

The pharmacological properties of Paludrine very closely resemble those of 4430, supporting the suggestion already advanced for 4430 (Spinks, 1946*b*) that the alkylbiguanide side-chain displays a conductophoric function analogous to that of the dialkylaminoalkyl-amino side-chain of mepacrine and related drugs. It will be the object of further work to decide from a study of related biguanides whether the inactivity of lower homologues of Paludrine is referable to a change in pharmacological properties, or whether the characteristics of the biguanide chain conferring activity are different from those conferring favourable pharmacology. Results so far obtained, particularly on the  $N_5$ -methyl homologue, indicate that the latter possibility is more likely.

## SUMMARY

1. Paludrine is rapidly absorbed in the rat, giving, however, only low concentrations

in blood and plasma, of the order of 2 and 0.75 mgm./l. respectively, following a single oral dose of 80 mgm./kgm.

2. Concentrations of Paludrine in the lung, liver, spleen and kidney of the rat are 10-50 times those in plasma.

3. Paludrine reaches higher concentrations in the mouse than in the rat following the oral administration of 80 mgm./kgm. It is similarly distributed in the two.

4. In the rabbit concentrations of Paludrine fall in the approximate order: kidney, lung, liver, spleen; heart, intestine, pancreas; muscle, fat; brain. The concentrations in red cells are 4-6 times those in plasma, in white cells probably 10-100 times those in plasma. These results have been compared with the corresponding ones obtained for 3349 and sulphaguanidine under the same conditions.

5. In the rat and the rabbit Paludrine is excreted mainly in the urine. Some drug reaches the intestine from the blood, in the bile and by other routes. The total recoveries in urine and faeces indicate that it is metabolized by both species.

**ACKNOWLEDGEMENTS.**—The author wishes to thank Miss R. B. Horrocks for technical assistance.

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# PHENAMIDINE IN THE TREATMENT OF *BABESIA BIGEMINA* INFECTIONS OF CATTLE

BY

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AND

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(Received for publication August 30th, 1946)

## INTRODUCTION

Lourie and Yorke (1939), in a survey of the action of certain aromatic diamidines on *Babesia canis* infections of puppies, showed that 4:4'-diamidino diphenyl ether (M. and B. 736) was both curative and non-toxic in action at a dosage of 10 mgm./kgm. given subcutaneously. Confirming this work, Carmichael (1942*a*, 1942*b*) pointed out the specificity and therapeutic efficiency of the drug in a single dose, the absence of undesirable reactions, and its low toxicity. Pierse (1943), using the more soluble dihydroxyethane-sulphonate (phenamidine isethionate), reported favourably upon its use in *B. bovis* infections of cattle in Northern Ireland, and Carmichael (1944) showed that it was effective against *B. caballi* in the only case treated.

## DATA

Twenty-four Zebu-Ankole cross-bred cattle, between one and two years old, which had been reared under conditions in which the tick-population had been reduced to a very low figure by dipping, were exposed for two weeks to *B. bigemina* infected ticks (*Boophilus annulatus* var. *decoloratus*) in a paddock which had previously held cases of babesiasis. Ten developed natural infections of *B. bigemina*, which were in all cases complicated by the presence of *Theileria mutans* and in one case by *Anaplasma marginale*. Nine of the cases were treated with phenamidine isethionate; details of the treated animals, and of the untreated control, are given in the accompanying table.

The drug was given subcutaneously; administration was not followed by any noticeable local reaction. In two cases which received phenamidine at 7.5 mgm./kgm., parasites were not eliminated, but subsequent treatment at 15 mgm./kgm. rapidly cleared up the infection. The drug at 15 mgm./kgm. and at 22.5 mgm./kgm. eliminated parasites from the blood within 48-72 hours, the length of time depending upon the severity of the infection and the level of dose. In all except the animal affected with *Anaplasma marginale*, clinical recovery was rapid.

The surviving animals were kept under close observation following treatment until February 27th, 1946, during which period there were no clinical relapses. They were then reintroduced into the original paddock and subjected to reinfection for a period of three months. B.5343 and B.5309 developed mild infections of *B. bigemina* on April 5th and 14th, 1946, respectively. Both infections were transient, and the animals recovered spontaneously.



## TOXICITY

The original 4:4'-diamidino diphenyl ether was shown to be markedly non-toxic for dogs by Carmichael (1944), who recorded that two or three times the curative dose

TABLE

Showing the course of infection with *B. bigemina* in nine bovines treated with phenamidine and in one untreated control

Bovine	Date	Temperature °F.	<i>B. bigemina</i>	<i>T. mutans</i>	<i>A. marginale</i>
5382	5.1.46	100.2	+	+	+
		Received phenamidine at 15 mgm./kgm.			
	6.1.46	102.0	+	+	+
	7.1.46	104.0	n.b.s.	+	+
	8.1.46	102.6	n.b.s.	+	+
	9.1.46	102.4	n.b.s.	+	+
	10.1.46	102.8	n.b.s.	v.s.	++
	11.1.46	100.8	n.b.s.	v.s.	++
	12.1.46	100.2	n.b.s.	—	+++
	13.1.46	95.0	n.b.s.	—	+++
	14.1.46	95.0	n.b.s.	+	+++
	15.1.46	Died			
5368	20.1.46	103.9	+	+	—
	21.1.46	104.0	+++	+	—
		Received phenamidine at 15 mgm./kgm.			
	22.1.46	103.0	++	+	—
	23.1.46	102.6	v.s.	+	—
	24.1.46	101.0	n.b.s.	+	—
	to 31.1.46		n.b.s.	+	—
5397	20.1.46	102.6	n.b.s.	++	—
	21.1.46	101.3	v.s.	+	—
	22.1.46	103.0	+	+	—
		Received phenamidine at 15 mgm./kgm.			
	23.1.46	102.4	v.s.	+	—
	24.1.46	98.6	n.b.s.	+	—
	to 31.1.46		n.b.s.	v.s.	—
5245	22.1.46	103.6	+++	+	—
		Received phenamidine at 15 mgm./kgm.			
	23.1.46	101.4	++	+	—
	24.1.46	98.6	+	+	—
	25.1.46	100.6	n.b.s.	+	—
	to 31.1.46		n.b.s.	+	—
5343	23.1.46	102.6	+	v.s.	—
	24.1.46	101.8	++	+	—
		Received phenamidine at 15 mgm./kgm.			
	25.1.46	100.6	v.s.	+	—
	26.1.46	100.2	n.b.s.	+	—
	to 31.1.46		n.b.s.	+	—
5350	25.1.46	103.8	++	—	—
		Received phenamidine at 7.5 mgm./kgm.			
	26.1.46	101.0	+	—	—
	27.1.46	100.2	v.s.	+	—
	28.1.46	99.7	v.s.	—	—
		Received phenamidine at 15 mgm./kgm.			
	29.1.46	98.6	n.b.s.	—	—
	to 7.2.46		n.b.s.	—	—

TABLE (Continued)

Bovine	Date	Temperature °F.	<i>B. bigemina</i>	<i>T. mutans</i>	<i>A. marginale</i>
5309	24.1.46	103·8	+	+	—
	25.1.46	104·0	+	+	—
	26.1.46	Received phenamidine at 7·5 mgm./kgm.	+	—	—
	27.1.46	100·8	+	+	—
	28.1.46	100·4	+	+	—
		100·0	+	+	—
	29.1.46	Received phenamidine at 15 mgm./kgm.	n.b.s.	v.s.	—
	30.1.46	99·0	n.b.s.	v.s.	—
	to 10.2.46		n.b.s.	+	—
5378	27.1.46	103·2	+	—	—
	28.1.46	102·6	+++	—	—
	29.1.46	99·8	+++	+	—
	30.1.46	Received phenamidine at 22·5 mgm./kgm.	+	+	—
	31.1.46	98·7	n.b.s.	+	—
	to 10.2.46	99·5	n.b.s.	+	—
			n.b.s.	+	—
5298	31.1.46	103·0	n.b.s.	+	—
	1.2.46	99·6	+	+	—
	2.2.46	101·2	+	v.s.	—
	3.2.46	Received phenamidine at 22·5 mgm./kgm.	n.b.s.	v.s.	—
	4.2.46	99·8	n.b.s.	v.s.	—
	to 14.2.46	99·4	n.b.s.	v.s.	—
			n.b.s.	v.s.	—
5344 : untreated control	27.1.46	103·4	+	+	—
	28.1.46	103·8	+++	+	—
	29.1.46	95·0	+++	+	—
	30.1.46	95·0	+++	+	—
	31.1.46	Died			

n.b.s. = No babesia seen after prolonged search of stained *thick* film.

v.s. = Very scanty. One parasite found after search of 100 fields of stained thin film.

+ = Parasites found after search of 20 fields in thin film.

++ = Parasites easily found in thin film.

+++ = Parasites present in every field of thin film.

of 10 mgm./kgm. produced no marked reactions. Phenamidine isethionate appears to be less well tolerated by cattle in large doses. Whilst the drug in a range of dose from 7·5 mgm./kgm. to 22·5 mgm./kgm. caused no toxic effects, of two healthy cattle which received 30 mgm./kgm. in 5 per cent. and 40 per cent. solutions subcutaneously, that which received the drug in the 40 per cent. solution died 18 days after administration. This result was unexpected, and the dose of 30 mgm./kgm. in 40 per cent. solution was repeated in a third animal; this died on the fourth day after receiving the drug. Post-mortem examination showed extensive liver damage in both cases.

## DISCUSSION

In the nine primary cases treated, phenamidine isethionate proved to be highly specific against *B. bigemina*. It is non-toxic and non-irritant for cattle in doses up to

22.5 mgm./kgm. The toxic effects observed when the drug was given at 30 mgm./kgm. in a 40 per cent. solution indicate that the dose should be accurately computed at the therapeutic level of 15 mgm./kgm.

It is unfortunate that no subinoculations were made from the animals following treatment, and it is impossible to state whether these cattle had actually been cured of the infection or whether they had been brought into a state of tolerance comparable with the natural spontaneous recovery seen in young indigenous cattle kept under native conditions of management. It is probable, however, that sterilization had in fact been produced, since there was no apparent reason why B.5343 and B.5309 should have become reinfected had they been in a stage of tolerance to the disease. Whether or not the animals were actually cured, it is evident that a considerable degree of immunity was developed following infection and subsequent treatment with phenamidine.

Lourie and Yorke (1939) and Fulton and Yorke (1941) demonstrated that *Babesia canis* could readily be made resistant to the related aromatic diamidines 4:4'-diamidino stilbene and 4:4'-diamidino diphenoxy propane, and that this resistance is considerable in degree and remains unchanged when the strain is passaged through a series of puppies. Whilst we have been unable to demonstrate drug-resistance in *B. bigemina* against the dihydroxyethane-sulphonate (largely owing to the fact that an infection induced by subinoculation is transient and mild), it would appear advisable that the drug be used at a dose of not less than 15 mgm./kgm. in order to obviate the possibility of producing drug-resistant strains.

It is noted that phenamidine has no effect upon *Theileria mutans* or *Anaplasma marginale*.

### SUMMARY

Phenamidine isethionate is remarkably specific in the treatment of *Babesia bigemina* infections of cattle at a dose of 15 mgm./kgm. The drug is well tolerated in doses up to 22.5 mgm./kgm. In view of possible toxic effects above this level, the dose should be accurately computed.

ACKNOWLEDGEMENTS.—Acknowledgements are due to Messrs. May and Baker Limited for supplies of phenamidine isethionate, and to the Director of Veterinary Services, Uganda, for permission to publish this paper.

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EDITORS' NOTE.—We are informed that, on November 30th, 1946, the eight animals which survived infection after receiving treatment with phenamidine were still under observation at the laboratory and that no relapses had been recorded.

# A MODIFICATION OF THE ZINC SULPHATE CENTRIFUGAL FLOTATION TECHNIQUE FOR THE CONCENTRATION OF HELMINTH OVA AND PROTOZOAN CYSTS IN FAECES

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(*Received for publication November 7th, 1946*)

The zinc sulphate centrifugal flotation technique of Faust and his associates (1938, 1939) has proved to be of such value in the concentration of helminth ova and protozoan cysts from faeces that it has very widely superseded the older and less efficient techniques for both clinical and research purposes. On account of its superiority in the revelation of protozoan cysts, particularly those of *Entamoeba*, it continues to be preferred to the simplified method introduced by Otto, Hewitt and Strahan (1941). Lane (1940) has criticized the method on the grounds that a loss of over 50 per cent. of helminth ova occurred when the specific gravity of the brine which he used for flotation fell from 1·3 to 1·2, and that consequently a zinc sulphate solution of specific gravity 1·18 might be expected to reveal less than half the ova in the sample. While admitting the validity of this criticism when quantitative work is involved, it must be remembered that Lane was concerned only with helminth ova, especially those of hookworm, and that the fact that zinc sulphate solution, unlike brine, is reliable for protozoan cysts as well as for helminth ova renders Faust's method invaluable as a routine qualitative diagnostic procedure. It has been in regular use as a diagnostic procedure in these laboratories over a considerable period of time for the daily examination of many faecal samples. In the course of this work, experience has suggested two slight modifications of the original technique which both simplify and improve it, and which, therefore, it may not be out of place to bring to the notice of a wider circle of laboratory workers.

In the first place, the process of sieving or screening the faecal sample to remove coarse particles is usually omitted, since tests with sieved and unsieved specimens have shown that the advantage to be gained is negligible in comparison with the labour and time involved. Coarse faecal particles are thrown down in the course of the centrifuging, while adult as well as larval worms are floated up in the zinc sulphate stage. Lane (1940) remarks that to strain the faecal suspension with the object of removing coarse particles may reduce the content of hookworm eggs by as much as 60 per cent., and he stresses the fact that the obvious advantages of a clearer preparation are overbalanced by this quantitative loss of ova. This being so, it is probable that straining may actually militate against detection of light infections — a very important matter in a general survey of incidence of intestinal protozoa and helminths.

In the second place, the use of a superimposed cover-slip, while admittedly superior to the use of a platinum loop or a fine pipette for removing the floating material from the surface film after final centrifugalization with zinc sulphate solution, is often avoided in practice, since it has the drawback of requiring a special bucket with projecting horns

to hold the cover-slip in position (Lane, 1924) or a special attachment to the metal tubes of the centrifuge in the form of a metal sleeve with four projecting fingers (Faust *et al.*, 1938). Faust and his fellow workers remarked that the superimposed cover-slip technique (modified D.C.F.) was abandoned because it required specially prepared Wassermann tubes and metal supports and was more laborious than other methods. Both they and Swartzwelder (1939) suggest 'touch-removal' of the surface film by applying a slide or cover-glass to the surface of the zinc sulphate solution, but this is admittedly less reliable than the use of the superimposed cover-glass. Thus, particularly in laboratories where the number of stool samples examined is small and where there is hence little incentive to have the necessary device added to the centrifuge, the superimposed cover-glass technique is not used as often as its efficiency demands.

In order to overcome this disadvantage it was decided to attempt to carry out the superimposed cover-glass technique without the assistance of special buckets or of the metal sleeve and fingers. As is usual, the glass centrifuge-tubes which were used projected slightly above the edge of the outer metal tubes and had been ground flat at the top. The cover-slip for superimposition, of slightly greater diameter than the glass centrifuge-tubes, was lightly smeared on one side with a thin film of Mayer's egg albumen (white of egg 50 c.cm., glycerine 50 c.cm., salicylate of soda 1 gm., shaken well together and filtered). After adding zinc sulphate solution in the usual way until the rim of the meniscus of the suspension was level with the top of the tube, the prepared cover-slip was pressed, prepared-surface downwards, on to the top of the tube. The sticky film of albumen against the rough-ground surface of the top of the tube is sufficient, when combined with the centrifugal force developed by spinning, to hold the cover-slip firmly in place. Clayton Lane (1922, 1924), in his original description of the superimposed cover-glass technique for centrifugal flotation methods, emphasized that the cover-glass must be held in place during centrifuging, otherwise it flies off. He tried the use of plasticine to keep the cover-glass in place, before resorting to the use of metal clips, but did not find it satisfactory. The extra-thick type of cover-glass advocated by Clayton Lane was not found to be necessary, since in the examination of many hundreds of stool samples by this method, using ordinary no. 1 cover-glasses, not one single breakage has occurred. Care must, however, be taken, in superimposing the cover-glass, to ensure that it is centrally placed in regard to the glass centrifuge-tube, so as to ensure that it is not knocked off as the metal bucket swings out on beginning to spin.

This modification has, further, two not inconsiderable advantages over the ordinary superimposed cover-glass technique, in that not only do cysts and ova thrown up tend to adhere to the sticky surface of the albumen film, and so are less readily displaced when the cover-slip is removed, but also that the albumen film provides an effective seal, so that there is no leakage of fluid between the top of the tube and the cover-slip, with consequent replacement by air, such as may sometimes occur with the ordinary method.

In conclusion, it may be useful to summarize briefly the sequence of operations in this modified zinc sulphate method as used in these laboratories:

1. A sample of the stool to be investigated, of about the size of a pea, is placed in the glass centrifuge-tube and broken up to form a fine suspension in distilled water by means of a thin wooden rod (orange stick).

2. The suspension is centrifuged for three minutes at 1,500 revolutions per minute, using an ordinary laboratory centrifuge with a radius of  $5\frac{1}{2}$  in. Supernatant fluid is then

removed and the process is repeated, usually twice, until the supernatant fluid at the end of the last spin is clear.

3. After removal of the clear supernatant fluid, zinc sulphate solution (33 per cent.  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ; specific gravity 1.18) is poured into the tube and the packed sediment is broken up into a uniform suspension. More solution is then added until the edge of the meniscus is level with the top of the glass tube.

4. A chemically clean circular cover-slip, of slightly greater diameter than the glass tube, is then lightly smeared on one side with a thin film of Mayer's egg albumen and pressed firmly and symmetrically on to the top of the glass centrifuge-tube, care being taken that no air-bubbles are trapped beneath it.

5. The suspension is again centrifuged for three minutes at 1,500 revolutions per minute.

6. When the centrifuge stops the superimposed cover-slip is carefully and rapidly lifted off the top of the tube and placed, prepared-surface downwards, on a drop of Weigert's iodine solution on a slide, when it is ready for examination.

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# THE EFFECT OF INTRAMUSCULAR INJECTION OF PALUDRINE AND MEPACRINE IN EXPERIMENTAL ANIMALS

BY

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## INTRODUCTION

Extensive experimental and clinical work with Paludrine has demonstrated its high activity as an antimalarial drug (see 'Studies on Synthetic Antimalarial Drugs,' 1945). In most cases Paludrine will be given orally, but sometimes it will need to be given parenterally. It must be emphasized that the drug is absorbed so rapidly that parenteral injections should not be given merely to achieve a rapid effect, and should be necessary only when the oral route is impossible. In these circumstances past work has shown that intravenous administration (100 mgm. is well tolerated) is the method of choice; but, since this may sometimes be difficult, the possible effects of intramuscular injection should be understood. These observations on experimental animals were made for that purpose.

Hawking (1943) drew attention to the lack of data regarding the local pathological effect of intramuscular injection of antimalarial substances. He pointed out that many clinical observations had been reported, and that, while some workers considered the local reaction to be unimportant, others—and this is quite well known—emphasized that, after intramuscular injection of mepacrine (atebrin) or quinine, there may be pain, injury and swelling, proceeding to abscess formation (the literature is given in Hawking's paper and need not be dealt with again here). Histological observations, however, had apparently never been carried out. Hawking studied the effect in rabbits and rats of intramuscular injections of mepacrine methanesulphonate (rabbits, 50 mgm. in 0.5 c.cm. distilled water; rats, 0.75 mgm.) and of quinine monohydrochloride. The rabbits were killed for examination after 1–5 days and the rats after 1–14 days. Macroscopic evidence of injury caused by mepacrine, like that caused by quinine, was considered to be usually slight, and the site of injection was often difficult to identify; the muscle was regarded as somewhat swollen, and small haemorrhages might be present after five days. Histologically, there were foci of necrosis, haemorrhage and degenerative changes of the nerve-trunks and larger blood-vessels. After 4–5 days a cellular reaction around the focus was more pronounced, and granulation tissue began to appear. A similar kind of lesion was produced by quinine monohydrochloride.

In our experiments, in addition to rabbits and rats, we have used larger animals, such as sheep, calves and dogs. This was done because it is clear that, following intramuscular injection, local damage will be proportional to the local concentration of the drug. If animal experiments, therefore, are to have any significance in relationship to the effects of the drug in treatment of malaria in man, the size of the dose must be at least equal to that which, it is contemplated, will be given to human cases.

## TECHNIQUE

The solutions used were 10 per cent. wt./vol. of Paludrine lactate and 15·25 per cent. wt./vol. solution of mepacrine methanesulphonate (both were calculated to contain 10 gm. of base). Sheep, calves, dogs and rabbits were used. The hair was clipped close to the skin, but not shaved, and prior to injection the area was cleansed with cetavlon; reasonable precautions were thus taken against sepsis. The sheep and calves were injected deep into the gluteal region, and the dogs and rabbits were injected into the posterior tibial group of muscles. The animals were killed at various intervals from 3 to 28 days, and a careful post-mortem examination was made of the site of inoculation, particular attention being given to evidence of possible local injury to the main nerve-trunks and vessels. The post-mortem examinations were conducted in a routine manner, attention being directed to any possible injurious effects in the viscera; but no lesions were seen in the latter.

The whole lesion at the site of intramuscular inoculation from each animal was fixed in 10 per cent. formol saline, and, after paraffin embedding, sections were stained with haematoxylin-eosin, Wilder's silver impregnation method and van Gieson for reticulum and connective tissue, and occasionally by orcein for elastic fibres.\* The sheep and calves were all between 100 and 150 lb. in body weight. Twelve sheep and five calves were given doses ranging from 50 to 400 mgm. of either Paludrine or mepacrine, and were killed after 3, 5, 12 and 28 days. Four dogs were given 100 mgm. of either drug and were killed after 5 and 12 days; one additional dog was injected with Paludrine in one leg and mepacrine in the other. Twenty-five rabbits were injected with 20, 40 and 50 mgm. of either substance and were killed at comparable periods.

## RESULTS

For convenience and brevity the results may be summarized by describing the average lesion as it was seen in the various animals at an early stage after three or five days, and at later stages after 12 and 28 days. These remarks can be applied to the effect of both Paludrine and mepacrine, since, apart from the yellow discoloration of tissue due to the mepacrine, there were no differences in kind or degree in the lesions produced and in the different species used.

There was no ulceration of the skin over the area of inoculation. Deep in the muscle there was a focus (in some sheep and calves between 2-8 cm. in diameter) which was pale, with marked haemorrhagic margins, with a minor degree of localized oedema, and occasionally with small semi-caseous necrotic points. In some the central necrosis was marked or the muscle had a cooked-meat appearance. In animals killed at later stages there was definite evidence of fibrous scarring. Histologically, sections of the skin over the site of inoculation showed an intact epidermis; the loose subcutaneous tissue below showed oedema, minute haemorrhages and a minor degree of inflammatory cell exudate, mainly polymorphs and histiocytes. The focus in the muscle was sharply delimited; the centre showed complete necrosis of muscle-fibres; all trace of sarcolemmal nuclei had disappeared (Plate II, fig. 1). Between the individual necrotic fibres and bundles of fibres there was oedema, evidenced by pale greyish-pink staining of homogeneous

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\* The full protocols are too comprehensive to be published; they can be consulted by application to the author.



material. The necrosis involved other structures in the area, viz., collagen, small vessels and nerves. At the margin of the lesion groups of muscle-fibres showed a greater affinity for eosin, and the myofibrils and sarcolemmal nuclei were partially broken up. The initial stage seemed to be the separation of the reticulin membrane from the sarcolemma. Haemorrhage was also prominent. In brief, the degree of damage faded as the periphery of the lesion was approached. Separating this area of trauma from normal muscle there was a wide irregular zone of inflammatory cell exudate, composed of a mixed collection of cells, including polymorphs, lymphocytes, a few eosinophils and many large macrophages (Plate II, figs. 1 and 2). There was marked evidence even at this stage of the occurrence of a highly active proliferating fibrous tissue—numerous fibroblasts, revascularization and the production of collagen fibres. The inflammatory response at the edge faded rapidly beyond into the muscle, in which normal structure was wholly intact. In animals killed at 12 days the lesion was of the above general nature, but of an extreme degree; necrosis and inflammatory reaction had apparently tracked along the fascial planes, along endomysial connective tissue and between bundles, forming a large irregular lesion; haemorrhage was extensive, and the fibroblastic reaction was more pronounced. At the margin some of the small arterioles and venules contained partially organized clots with adhesions to the wall, and there was much periadventitial fibrosis. Some of the degenerated muscle-fibres contained dark-staining basophilic granules, which might have been due to the breakdown of myoglobin. At a later stage (sheep and calves injected with 100 mgm. and killed at 28 days) the muscular lesion was in the form of a dense fibrotic scar, with a gross production of thick intertwining collagen fibres and occasional areas of calcification, while the inflammatory cell infiltration was of a minor degree (Plate II, fig. 3). Scattered throughout the scar were remains of degenerated muscle-fibres.

## DISCUSSION

There was abundant evidence of severe macroscopic and histological injury in nearly all the animals following the intramuscular injection of either Paludrine lactate or mepacrine methanesulphonate. Some of the doses given to larger animals would approximate to that which might be given to man, viz., 50–100 mgm. to sheep weighing 150 lb. The doses given to dog and rabbit were, no doubt, high, but altogether the experiments formed a very stringent test regarding possible intramuscular injury if the drug was injected in human cases at a dosage level of between 50 and 100 mgm. The lesion produced was essentially that of a sharply delimited, large, irregular focus of myonecrosis, with all the concomitant sequelae which might be expected to follow as a local effect, on subcutaneous and muscular tissue, of a high concentration of a relatively toxic substance, viz., haemorrhage, oedema, polymorphous inflammatory cell exudate, and, as the concentration of the drug disperses and repair takes place, a very active fibroblastic proliferation and the production of scar tissue. The trauma also involved small vessels and nerves present in the site; the production of thrombosis was noteworthy. There was little difference in kind or degree between the effect of Paludrine and mepacrine, except that in the group of *rabbits* injected with 20–40 mgm. mepacrine there was little macroscopic evidence of injury and histologically the reaction was more mild. In general, the damage was such that it must be accompanied by pain, particularly if the site of inoculation involved nerves. The lesions produced were obviously due to the effect of the drug



FIG. 1. Effect of intramuscular injection of Paludrine lactate. 400 mgm. Sheep. Killed after 5 days. Margin of lesion, normal muscle-fibres, bottom right. Remnants of necrotic muscle-fibres above, amidst oedematous fluid; haemorrhage; inflammatory cell exudate. H.E.  $\times 92$ . (190.46A.)

FIG. 2. Effect of intramuscular injection of Paludrine lactate. 50 mgm. Sheep. Killed after 3 days. Margin of lesion starting bottom left. Marked necrosis, fragmentation and liquefaction of muscle-fibres, with intense haemorrhage, inflammatory cell infiltration and exudate between fibres. H.E.  $\times 92$ . (222.46G.)

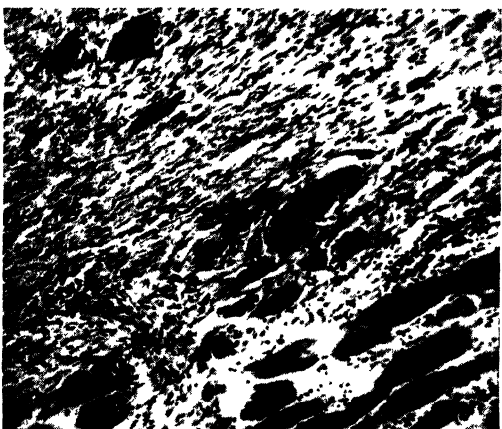
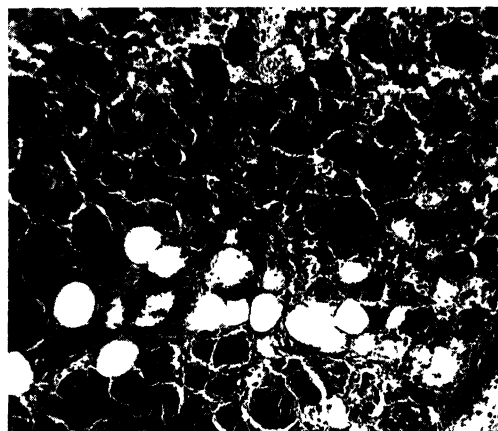


FIG. 3. Effect of intramuscular injection of Paludrine lactate. 100 mgm. Sheep. Killed after 28 days. (The residual lesion was a fibrous scar in muscle, 2 cm. in diameter.) Margin of lesion bottom right, showing remains of normal fibres, in which striation is still evident; lesion passes into area of intense fibrosis, with an occasional fragmented muscle-fibre. H.E.  $\times 92$ . (222.46a.)



and not to any complication of secondary infection introduced along the track of the needle.

Both drugs thus produce marked local damage at the site of intramuscular injection. The doses which have been used are at least as big as those which would be given to human beings, and it seems permissible to draw the following conclusions.

1. There is some justification for the statement made by Hawking (1943) that mepacrine should not be injected by any route unless it was impracticable to give it by mouth—to which we might now add that the same applies to the intramuscular injection of Paludrine.

2. Whatever the therapeutic dose, intramuscular injection is certain to cause some trauma, followed by myonecrosis, inflammation and repair ; as a result the injection is likely to be somewhat painful.

3. If the usual precautionary measures are taken regarding sterility, no ulceration should follow.

4. It is clear that precautions should be taken to avoid the careless injection of the drug anywhere near the course of nerves or vessels.

5. The initial lesion is followed by fibrosis, which will lead to the production of a small scar, but no permanent damage of any significance should result.

### SUMMARY

1. The effect of intramuscular injections of Paludrine and mepacrine in experimental animals—sheep, calves, dogs and rabbits—has been examined.

2. Both drugs, at doses comparable to those which might be given in human cases of malaria, produce a marked focus of myonecrosis and inflammatory reaction followed by fibrosis.

3. The lesions produced would appear to depend upon the local concentration of drug.

4. In the case of Paludrine, intramuscular injection should seldom be necessary, and only when it is impracticable to give the drug by mouth or by intravenous injection.

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# INCUBATION OF TSETSE PUPAE: THE TEMPERATURE RANGE EXPERIENCED BY PUPAE KEPT UNDER NORMAL LABORATORY CONDITIONS AT TINDE\*

BY

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In transmission-experiments with *Glossina morsitans* and *Trypanosoma rhodesiense* at Tinde laboratory (Burtt, 1946), it was found that better transmissibility and higher infection-rates occurred in flies which emerged from pupae incubated at approximately 30° C. than in flies from pupae kept at normal laboratory temperatures. By an oversight these normal laboratory temperatures were not given, and they are accordingly presented herewith. Pupae were kept in wide-mouthed bottles closed with mosquito-netting standing inverted on wire gauze over water in a zinc tray resting on a table. From March, 1943, a slight modification was made in that the zinc tray was thenceforth kept in a closely fitting open wooden box with sides projecting about five inches above the contained tray. This possibly made temperature and humidity conditions on the tray slightly more uniform.

Records of maximum and minimum temperatures from the surface of wire gauze in an open zinc tray containing water were started in August, 1941, and continued to July, 1942. A break in the recording then occurred until March, 1943, after which the temperatures were taken from the open wooden box, as mentioned above. Hicks maximum and minimum thermometers were used. The mean daily maximum and median temperatures by months are given in the accompanying table. The median temperatures have been given to facilitate comparison with the other pupae which were being incubated at approximately 30° C.

TABLE  
Mean daily maximum and median temperatures by months at Tinde Laboratory

	On wire gauze over water in zinc tray				As on left, but zinc tray in open wooden box					
	1941		1942		1943		1944		1945	
	Maximum	Median	Maximum	Median	Maximum	Median	Maximum	Median	Maximum	Median
January ...	—	—	28.5° C.	24.8° C.	—	—	29.9° C.	25.9° C.	29.3° C.	24.3° C.
February ...	—	—	29.6 "	25.6 "	—	—	29.3 "	25.1 "	28.8 "	23.9 "
March ...	—	—	27.4 "	24.2 "	28.7° C.	25.7° C.	29.3 "	25.1 "	29.7 "	24.5 "
April ...	—	—	27.6 "	24.1 "	28.6 "	25.4 "	27.6 "	23.8 "	29.7 "	26.1 "
May ...	—	—	27.1 "	23.3 "	27.6 "	24.6 "	28.2 "	24.0 "	28.3 "	24.8 "
June ...	—	—	27.6 "	23.2 "	26.9 "	23.5 "	28.4 "	23.7 "	—	—
July ...	—	—	25.9 "	21.7 "	26.9 "	23.1 "	27.6 "	22.9 "	—	—
August ...	28.9° C.	25.0° C.	27.4 "	23.0 "	28.4 "	24.5 "	28.5 "	23.7 "	—	—
September ...	30.4 "	26.3 "	—	—	30.1 "	26.0 "	29.8 "	24.9 "	—	—
October ...	31.9 "	28.1 "	—	—	31.3 "	27.1 "	31.0 "	25.8 "	—	—
November ...	28.3 "	24.9 "	—	—	31.1 "	27.1 "	29.4 "	24.6 "	—	—
December ...	28.0 "	24.5 "	—	—	31.9 "	27.7 "	29.7 "	24.6 "	—	—

\* Addendum to an earlier paper (Burtt, 1946).

The table shows that, at Tinde laboratory, the mean daily maximum temperature, reckoned by months, sometimes reached or slightly exceeded 30° C. during the period September to December. This does not mean that temperatures of over 30° C. did not occur during the remainder of the year, but they were comparatively infrequent. Circumstances prevented continuous temperature records being kept, so that the number of hours that the temperature remained above 30° C. on a hot day is not known. Comparison with records\* kept at the Tsetse Research Laboratory, Shinyanga, 26 miles distant, indicate that during the hottest period (August-December) a temperature of over 30° C. was experienced in the laboratory for usually from one to seven hours—about five hours being the most frequent period; during other shorter hot spells about two hours was usual.

It will be seen from the table that the median temperature at Tinde laboratory was always below 30° C., by values ranging from about 2° C. during the hottest to over 8° C. during the coolest months.

Thus the incubation of pupae at approximately 30° C. did not entail their being subjected to temperatures entirely outside the range experienced by those kept under normal laboratory conditions—particularly during the hot season; but, whereas the latter were only subjected to such temperatures for spells of a few hours at a time (and always to considerably lower temperatures at night), the incubated pupae were experiencing the higher temperature continuously.

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\* The writer is indebted to the Acting Director of the Tsetse Research Department for making these records available to him.

# AORTIC SIZE IN EAST AFRICAN NATIVES\*

BY

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Few standard measurements are as yet available of the weights and sizes of the internal organs of East African natives. During the recent war I made notes of the weights and measurements of the internal organs of askaris brought for post-mortem examination. Many of the findings are comparable with standard European weights and measurements, but the circumference of the East African's aorta is so significantly different from European standards that the data are worth recording.

## MATERIAL AND METHOD

The material on which this study was based were the aortas of 60 Africans who had died from surgical conditions, accidents or acute illnesses. A certain selection was exercised, in that measurements were not made on any cadaver showing signs of cardiovascular or renal disease. Only aortas from askaris of estimated age of 20-30 years were measured. The following measurements, therefore, represent, as far as possible, measurements of the aortas of young East African males of tribes enlisted in the East African army.

The thoracic aorta was detached *in toto* and then split open longitudinally. The circumference was measured by laying a steel rule on the opened surface of the aorta, at the level of the origin of the fourth intercostal artery. Measurements were made to the nearest millimetre. This is precisely the method which was used by Millar and Ross (1942) in measuring aortic circumferences in Europeans. Actually, the level at which the measurement is made matters little, as the thoracic aorta is practically cylindrical, and in no case was there a difference of as much as 1 mm. between the measurements in the upper and the lower parts of the thoracic aorta.

TABLE I

Showing the circumferences of thoracic aortas in East African males

Circumference, in mm. ...	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
No. of times recorded ...	1	0	1	4	3	7	4	9	7	7	7	3	4	0	0	3

Table I shows the measurements found in 60 aortas. The average circumference was 43 mm. (the median and the mode = 43 mm. ; the geometric mean = 42 ;  $\sigma = 3.2$  ; S.E. $\sigma = 0.3$ ).

\* These observations were carried out while the author was attached to a military general hospital in Nairobi.

## DISCUSSION

Millar and Ross (1942), in Edinburgh, carried out a series of measurements on aortic size in 300 consecutive post-mortems. The mean aortic circumference of these 300 cases was  $52.34 \pm 0.44$  mm. before any corrections for age, weight, height and sex had been made. The adjusted value recorded was  $52.40 \pm 0.23$  mm. Of these 300 cases analysed, 26 had died of accident, and the mean value of the adjusted aortic circumference was  $51.05 \pm 0.49$  mm.; the adjusted circumference of the other 274 cases was  $52.53 \pm 0.25$  mm. Stitt, Clough and Clough (1945) give the average aortic diameter as 1.7-3 cm., which gives a calculated circumference of 53-94 mm.

It can be seen that the mean circumference of the African's aorta is about 9 mm. less than the mean value recorded in Millar and Ross's 300 cases, and that the greatest circumference of any recorded aortic measurement in the East African (50 mm.) is 3 mm. less than the lowest figure (53 mm.) given in Stitt, Clough and Clough's range of standards.

TABLE II

Showing measurements of aortic circumference, in mm., in East African natives compared with other standards

Race ...	Africans (present series)	Europeans (Millar and Ross)	Not stated (Stitt, Clough and Clough)
Range ...	35-50	—	53-94
Mean ...	43	$52.34 \pm 0.44$ (before correction)	—

It should be noted that the bodily size and shape of the cadavers of the Africans whose aortas were measured varied about as much as they would among a similar random sample of Europeans. In all cases the Africans had fulfilled the necessary standards of height, etc., for enlistment into the army.

A hypoplastic vascular system is said to be one of the macroscopic characteristics of the *status thymo-lymphaticus*. Whether or not there is such a pathological entity as the *status thymo-lymphaticus*, it is the rule rather than the exception to find an unduly persistent thymus gland in performing autopsies on young African adult males, and it may well be that in East African natives there is some connection between the persistent thymus and an abnormally narrow aorta.

Bedford (1946) has observed that, in a number of cases of sudden heart-failure in Africans, autopsy showed that they had markedly hypoplastic aortas, and on examining X-ray films of Africans' chests he found evidence of a small aorta in a big proportion of them.

The present series and other measurements of internal organs of East African natives serve to show how different the African body is from that of the European, and perhaps will help to guard against the too prevalent tendency to apply European standards to Africans before more intimate knowledge of their anatomy and physiology is known.

## SUMMARY

1. The circumference of the thoracic aorta of East African natives is significantly smaller than the standard measurements of aortic circumference in Europeans.



2. The persistent thymus found at autopsy in many East African natives may be related to a hypoplastic vascular system.

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# THE LIFE-CYCLE AND HABITS OF *CULICOIDES IMPUNCTATUS* GOETGHEBUER AND *CULICOIDES OBSOLETUS* MEIGEN, TOGETHER WITH SOME OBSERVATIONS ON THE LIFE-CYCLE OF *CULICOIDES ODIBILIS* AUSTEN, *CULICOIDES PALLIDICORNIS* KIEFFER, *CULICOIDES CUBITALIS* EDWARDS AND *CULICOIDES CHIOPTERUS* MEIGEN

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## I. INTRODUCTION

### (1) THE DISTRIBUTION OF THE GENUS *Culicoides*

The small blood-sucking Diptera of the genus *Culicoides* appear to occur in all tropical and temperate zones of the world except New Zealand (Macfie, 1932), Patagonia and Southern Chile (Ingram and Macfie, 1931). They are known colloquially by a variety of names, such as 'midges' in Great Britain, 'punkies' or 'sandflies' in the United States, and 'jejenes' in Spanish-American countries.

### (2) THE IMPORTANCE OF THE GENUS

(a) *Nuisance Value.* Wherever *Culicoides* occur the irritation and annoyance caused by their bites is often so great as to constitute a major pest. This nuisance may reach such proportions as seriously to interfere with tourist trade at seaside resorts. Certainly the tourist business in the Highlands of Scotland suffers severely through the activities of certain species of *Culicoides*, and in order to try to improve these conditions the Scientific Advisory Committee of the Secretary of State for Scotland appointed a sub-committee in January, 1945, to suggest methods for the control of these annoying insects. In some countries the numbers of *Culicoides* are sufficient to make outdoor work impossible, and it has been suggested that these biting insects are largely responsible

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for the slow development of the southern areas of the Atlantic seaboard of the United States (Dove, Hall and Hull, 1932). The view has also been expressed (Edwards, 1939) that a similar state of affairs exists in some parts of Great Britain, and that in Western Scotland the backward state of croft-farming may, in part, be attributed to their activities.

Not only man but other mammals and also birds (Jellison and Philip, 1933) are attacked by *Culicoides*, and according to a report from the Bahamas even lizards are not immune from their bites (Myers, 1934). One Oriental species is also reported to attack earthworms in great numbers (Patel, 1921) and another to obtain its blood-meal from gorged mosquitoes (Sinton and Little, 1925).

(b) *Transmission of Disease.* The chief significance, however, of *Culicoides* is as a vector of disease, particularly of filariasis, which in the tropics it conveys both to man and to his domestic stock of cattle and horses; while in the temperate zones, including Great Britain, although human cases are unknown, horses are infected.

As regards human filariasis, it was established by Sharp (1928) that *C. austeni* Carter is a vector of the worm *Filaria perstans* Manson in West Africa and that *C. grahami* Austen is a probable vector. Buckley (1934), in St. Vincent, observed the complete development of the larvae of *Filaria ozzardi* Manson in females of *C. furens* Poey fed on infected persons. At about the same time Dampf (1936) in Mexico found *C. filariferus* Hoffman infected with filariae in the sausage stage, but the identity of these immature forms was not proved, although they suggest that some belonged to a species of *Onchocerca* which attacks man—recorded as possibly *O. volvulus* (Leuckart, 1893)—others to a species parasitizing equines and possibly cattle.

Concerning filariasis of animals, Steward (1933), working in this country, showed that the worm *O. cervicalis* Railliet and Henry is transmitted by *C. nubeculosus* Mg. and probably by *C. obsoletus* Mg. and *C. parroti* Kieff. Five years later Buckley (1938) demonstrated that *Culicoides* is a vector of *O. gibsoni* Cleland and Johnston to cattle in Kuala Lumpur. It has also been shown that certain species of *Culicoides* are capable of transmitting filariasis of monkeys in Panama (see Causey, 1938).

In addition to filariasis, *Culicoides* transmit pathogenic virus infections to domestic stock. Du Toit (1944), in South Africa, recorded three positive infections of sheep with blue-tongue and one positive infection of a horse with horse-sickness following intravenous injection with emulsions of *Culicoides* caught in the field; in addition, one sheep developed blue-tongue after being bitten by *Culicoides* that had fed 10 days previously on infected sheep. In Formosa a species of *Culicoides* is incriminated as causing fowlpox of domestic fowls and turkeys, the midge breeding in the dung of the poultry which it attacks (Tokunaga, 1937).

In view of the importance of these insects as pests and as vectors of disease, it is surprising to find that there is no account of the complete life-cycle of a single British species in the literature, nor, with one exception—*C. oxystoma* Kieff.—of a foreign species. Moreover, whereas there are many scattered statements concerning the abundance and breeding-sites of different tropical and temperate species, there is little information available regarding the length of their life-cycles or the seasonal abundance of the larvae and adults.

Without full knowledge of the life-cycle and considerable knowledge of the peaks of seasonal abundance, mere records of the distribution, breeding-sites and relative densities of different species cannot afford the information necessary for control-methods

to be applied to the best advantage. It was in order to supply some of this information that the present investigation, which was undertaken in the neighbourhood of Liverpool, was instigated. It includes observations on two of the most universally annoying species of *Culicoides* in the British Isles, namely *C. impunctatus* Goet. and *C. obsoletus* Mg., and on the less widely distributed species *C. odibilis* Austen, *C. pallidicornis* Kieff., *C. cubitalis* Edw. and *C. chiopterus* Mg.

## II. SURVEY OF LITERATURE

### (1) GENERAL SURVEY OF EXISTING KNOWLEDGE CONCERNING THE LIFE-CYCLE OF *Culicoides*, WITH PARTICULAR ATTENTION TO THE LENGTH OF THE CYCLE

Over two centuries ago, in 1712, the Rev. W. Derham, then Rector of Upminster in Essex, gave the earliest description of the life-history of a species of *Culicoides*, the larvae and pupae of which live in water, during a course of 16 sermons sponsored by a certain Mr. Boyle and given in St. Mary-le-Bow Church, London. The substance of these sermons was published in a book written by Derham (1713) entitled *Physico-Theology: or a Demonstration of the Being and Attributes of God from His Works of Creation*. The following account of the life-history of *Culicoides* is quoted from this volume.

‘For an Instance of Insects endued with a Spear, I shall, for its peculiarity, pitch upon one of the smallest, if not the very smallest of all of the *Gnat*-kind, which I call *Culex minimus nigricans maculatus sanguisuga*. Among us in *Essex* they are called *Nidiots*, by *Mouffet*\* *Midges*. It is about 1/10 of an inch, or somewhat more long with short *antennae*, plain in the female, in the male feather’d, somewhat like a Bottle-brush. It is spotted with blackish spots, especially on the wings, which extend a little beyond the Body. It comes from a little slender Eel-like Worm, of a dirty white Colour, swimming in stagnating Waters by a wriggling Motion. . . .

‘Its *Aurelia* [pupa] is small, with a black Head, little short Horns, a spotted, slender rough Belly. . . . It lies quietly on the top of the Water, now and then gently wagging itself this way, and that.

‘These Gnats are greedy Blood-suckers, and very troublesome where numerous, as they are in some places near the *Thames*, particularly in the Breach-waters that have lately befallen near us, in the Parish of Dagenham; where I found them so vexatious, that I was glad to get out of those Marshes. Yea, I have seen Horses so stung with them, that they have had drops of Blood all over their Bodies, where they were wounded by them.

‘I have given a figure . . . and more particular description of the gnats because, although it be common, it is nowhere taken notice of by any author I know, except *Mouffet*, who, I suppose, means these gnats, which he calls midges.’

Linnaeus (1790) records the species described by Derham as *C. pulicaris* L., but it was not necessarily the same species as to-day bears that name, since apparently at that time only one species of *Culicoides* was recognized in Britain.

Two centuries passed before a further description was given of the life-history and habits of *Culicoides*, when Lutz (1912) published an account of the life-history and habits of several Brazilian species of *Culicoides* which he found breeding in the mud of crab-holes on the margin of a mangrove swamp.

The following year Patton (1913) published an illustrated account of the stages in the life-history of an Indian species, *C. pattoni* Kieff. syn. *C. kiefferi* Patton.

Since the work of these earlier writers many authors have given incomplete accounts

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\* Derham's reference is to *Mouffet's* book *Insectorum sive Minimorum Animalium Theatrum* (1634), of which the original manuscript, with two dedications, is still preserved among the Sloane MSS. in the British Museum. This book is said to be the first zoological work published in England and contains a reference to small blood-sucking gnats popularly known as ‘midges’ in England.

of the life-cycle of various species of *Culicoides*. These include Goetghebuer's (1919) description of *C. pulicaris* in Belgium, the account of Carter, Ingram and Macfie (1920) of a number of African species, and the description given by Patel (1921) of the habits and the life-cycle of *C. oxystoma* in India. Our scanty knowledge of the life-histories of European species has been summarized by Rieth (1922), Thienemann (1928) and Mayer (1934a).

The present survey of the literature concerning the bionomics of *Culicoides* has so far been of a general character, but, since a number of workers have paid special attention to the length of the life-cycle, and since it has proved of particular importance in our own work, we propose to consider it separately and in some detail.

According to the literature, the length of the life-cycle of *Culicoides* varies with climatic conditions and with the species. The length of the pupal stage is generally given as from three to seven days, but the writers' statements concerning the length of the larval stage vary considerably.

Buckley (1938), who located breeding-places of *Culicoides* in Kuala Lumpur by taking samples of mud and placing them in gauze-covered cylinders, states that any eggs or larvae in the material became adults within 1-4 days—a surprising result in view of the findings of other authors. Thus, Carter, Ingram and Macfie (1920), who worked on certain West African species of *Culicoides*, state that 'The duration of the larval stage was not determined in any of our species, but it is probably largely influenced by the food supply, and may certainly extend over several weeks. The duration of the pupal stage is from three to five days.' Patel (1921) worked out the life-history of *C. oxystoma* in captivity and records that under these conditions the egg stage of this species lasts from three to 11 days, the larval stage from two to 10 weeks, and the pupal stage from three to seven days. Dove, Hall and Hull (1932), writing of *C. canithorax* Hoffman and *C. dovei* Hall, both Florida salt-marsh species, state that 'Larvae more than one-half grown were kept in the laboratory for periods varying from four to six and one-half months . . . some of each species pupated and produced adults during the spring months . . . larval development may last at least six months, if not an entire year. . . . The pupal period for sand flies lasts for only four to seven days.' That the cycle of certain species may be relatively short, although never as brief as that observed by Buckley, is suggested by the work of Tokunaga (1937), who states that *C. sugomotonis* Shiraki, which attacks fowls and turkeys, appears to have more than 10 generations in a year, while Kinoshita reported (Tokunaga, 1937) that emergence of adults of *C. milarai* Kinoshita, a biting shore midge from Korea, occurs twice a year, the first emergence occurring from the beginning to the end of June and the second from the beginning of August to the middle of September.

All the authors so far quoted have worked with species of *Culicoides* which are not found in Great Britain. The first statement on the length of the life-history of a species which occurs in this country was made by Goetghebuer (1919), who writes of *C. pulicaris* that it has not yet been ascertained in what stage the winter is passed, that there are several generations a year, and that the duration of the larval stage is unknown. The life-history of *C. vexans* Staeg., another British species, has been investigated by Jobling (1929), who summarizes his findings thus: 'It breeds in damp earth round bushes and under big trees, where desiccation of the ground is prevented by overhanging branches. The midge has only one generation a year. From eggs laid in June and kept in the open, the first stage larvae hatched in October. Further observations on these larvae were then

made in the laboratory, and in the following March many midges of both sexes were hatched. . . . 'The pupal stage is of very short duration lasting only a few days.' The only other record of the life-history of a British species of *Culicoides* is that of Steward (private communication), who kept *C. nubeculosus* in the laboratory. From a single batch of eggs laid in the first week of August, 1932, one male hatched on October 9th. No further emergences occurred until the following year, when 60 per cent. of the larvae pupated between April 20th and 25th, the adults beginning to emerge a few days later.

Medwedewa (1927) states that the majority of larvae of *C. nubeculosus* collected in July pupated in August, September and October when kept at room-temperature, which probably reduced the length of the developmental period considerably. Under natural conditions he states that these larvae may pass right through the winter and pupate in the spring. This would imply that the species observed by Medwedewa has one generation per year. However, since this species lived in brackish water, it is possible that it was *C. riethi* Kieff. and not *C. nubeculosus*. It also appears to us that Medwedewa's drawing of the male genitalia more nearly approximates to *C. riethi* than to *C. nubeculosus*.

With regard to *C. obsoletus*, Edwards (1939) writes: 'Though most abundant in June, *C. obsoletus* may be found throughout the summer, and in a damp autumn is sometimes very numerous again in September and October, a fact which suggests that it has normally at least two broods in the year.' Of *C. impunctatus* he states that nothing is known of the length of the life-cycle.

## (2) GENERAL SURVEY OF EXISTING KNOWLEDGE CONCERNING THE MORPHOLOGY OF THE ADULT AND IMMATURE STAGES OF *Culicoides*

The references so far given apply only to papers dealing with the bionomics of the genus *Culicoides*. In contrast to the paucity of knowledge concerning the bionomics, a considerable literature dealing with the systematics of this genus—most of which is concerned only with the adult forms—has grown up during the same period. It is impossible to review the whole of the literature concerning the adult forms here, and we propose to give only the principal references concerning the existing knowledge of the European species. Excellent accounts of these are to be found in papers written by Edwards (1926, 1939), Goetghebuer (1920, 1933), Goetghebuer and Lenz (1933) and Kieffer (1919, 1925).

The situation regarding the literature dealing with the morphology of the immature stages is unfortunately less satisfactory, and, in order to save others a fruitless search for information concerning the immature stages of any species in which they may be interested, it seems desirable to summarize briefly those relatively few papers which the present writer has found to contain any account of this subject.

The first general description of the morphology of the larva and pupa of *Culicoides* is that of Carter, Ingram and Macfie (1920). This excellent and well-illustrated account is part of their study of certain West African species and includes descriptions of the larvae and pupae of *C. inornatipennis* Carter, Ingram and Macfie, *C. fulvithorax* (Austen), *C. schultzei* (End.), *C. punctithorax* Carter, Ingram and Macfie, *C. accraensis* Carter, Ingram and Macfie, *C. clarkei* Carter, Ingram and Macfie, *C. eriodendroni* Carter, Ingram and Macfie, and *C. nigripennis* Carter, Ingram and Macfie, and a description of the larva of *C. punctithorax* Carter, Ingram and Macfie. The only other description of the

immature stages of a tropical species of *Culicoides* is that of Patton (1913), who gives some account of the morphology of *C. pattoni*, an Indian species.

These are the only references which the present writer has traced concerning the morphology of the immature stages of tropical species of *Culicoides*. Our knowledge of the morphology of the immature stages of European *Culicoides* is scanty, but descriptions of, and keys to, certain species have been written.\* Rieth (1922) gives a description, with figures, of the larvae and pupae of *C. setosinervis* Kieff., *C. riethi*, *C. stigmatus* Kieff. and *C. salinarius* Kieff., and of the pupae of *C. festivipennis* Kieff. and *C. pulicaris*. He also indicates the breeding-places of these species.

Thienemann (1928) gives a key to the larvae of *C. pulicaris*, *C. rivicola* Kieff., *C. salinarius*, *C. punctatidorsum* Kieff. (*C. salinarius* Kieff.), *C. salicola* Kieff. and *C. riethi*, and a key to the pupae of *C. rivicola*, *C. pullatus* Kieff., *C. festivipennis*, *C. pictipennis* Staeg., *C. crassiforceps* Kieff., *C. riethi*, *C. nubeculosus*, *C. algarum* Kieff., *C. salinarius*, *C. salicola*, *C. punctatidorsum* and *C. meinerti* Kieff. Figures are also given of the opercula of *C. rivicola*, *C. stigmatus* and *C. salinarius*.

Tokunaga (1937) describes in detail the larva of *C. circumscriptus* Kieff., while Lenz (1934), combining the keys of other workers, gives a key to the known pupae of European species. This key includes *C. nubeculosus*, *C. pulicaris*, *C. setosinervis*, *C. stigmatus*, *C. halophilus*, *C. winnertzi* Ed. syn. *C. festivipennis* and *C. odibilis*, *C. pictipennis*, *C. crassiforceps*, *C. nubeculosus* syn. *C. riethi*, *C. algarum*, *C. salinarius*, *C. punctatidorsum* and *C. meinerti*.

Mayer (1934a) records the breeding-places of numerous European species and gives diagrams of the operculum and anal segment of *C. obsoletus* and of the breathing-trumpet of *C. rivicola*. This author also describes the typical pattern of the tubercles on the third abdominal segment of *Culicoides* pupae and gives the pattern of these tubercles for *C. rivicola* and *C. nubeculosus*. In addition, he indicates that the pharyngeal skeleton might be used in larval differentiation and gives figures of the pharyngeal skeleton of *C. nubeculosus* and a species purported to be *C. rivicola*. Previously Carter, Ingram and Macfie (1920) had indicated that the pharyngeal skeleton might prove to be an important feature in larval differentiation.

This survey of the literature reveals that, of the species under consideration in the present paper, only the immature stages of *C. obsoletus* (of which *C. rivicola* is now regarded a synonym) have been described, and that the description of the larvae of this species is inadequate.

### (3) SURVEY OF EXISTING KNOWLEDGE CONCERNING THE MORPHOLOGY, DISTRIBUTION, LIFE-CYCLE AND HABITS OF THE BRITISH SPECIES OF *Culicoides* CONSIDERED IN THE PRESENT INVESTIGATION: (a) *C. impunctatus*, (b) *C. obsoletus*, (c) *C. odibilis*, (d) *C. pallidicornis*, (e) *C. cubitalis*, (f) *C. chiopterus*

(a) *C. impunctatus* was first described from Belgium by Goetghebuer (1920), who considered only the female, the male genitalia not being described or illustrated until Edwards (1939) published an account of the male genitalia of the known British species. More recently Tokunaga (1941) has given a more complete description of the female of the species.

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\* These are listed under the names used by the authors concerned. The synonyms recognized to-day have not been substituted.

The records indicate that this species is not so widely distributed as *C. obsoletus*, but it is wide-spread in Europe (Mandoul, 1929) and Tokunaga (1941) records it from Manchuria. In Great Britain *C. impunctatus* appears to have a wide range: the British Museum collection includes specimens from Brodick, Catacol and Pirnmill (Arran), Lochinver and Loch Assynt (Sutherland), Loch Morlich (Inverness), Loch Callater (Aberdeen), Witherslack, Brother's Water and Staveley (Westmorland), Penshaw (Durham), Malham Tarn (Yorkshire), Sandy (Bedfordshire), Bracknell (Buckinghamshire), Epping Forest (Essex), Goathorn (Dorset), Gorge of Dart, Gidleigh and Dartmouth (South Devon), Ffrith (Flintshire), Pant-Clyd (Merioneth), Crymlyn Bog (Glamorgan), and Killarney (South-West Ireland); and Cameron *et al.* (1946) record it from Sutherland, Ross and Cromarty, Inverness, Argyll, Perth, Stirling, Dumbarton, Renfrew, Lanark, Dumfries, Peebles, Midlothian, Angus, Kincardine and Aberdeen, but they state that the great majority of their large collections came from the Highlands.

The date of capture, as recorded in the British Museum collection of *C. impunctatus*, ranges from the end of May to September, while that of the specimens captured by Tokunaga in Manchuria ranges from June to the beginning of August. There appears to be no record in the literature concerning the immature stages of this species, except that of Goetghebuer (1936), who records it as breeding in peat-bogs, but there is a little information regarding the habits of the adults. Goetghebuer (1935) states that it attacks man at night as well as in the daytime, while Edwards (1921) records it (as *C. arcuatus* Winn.) in swarms after 6 p.m. (summer time) and mentions that it will bite all through the night in sultry weather (Edwards, 1939). Cameron (1946), writing of the Scottish midges, of which by far the most prevalent species is *C. impunctatus*, states that 'Wide-spread and severe attacks do not usually occur until the middle of June, but midges may bite in small numbers before then. The worst months are July and August, the first fortnight of the latter month usually being especially bad. The midges normally disappear for the season at the end of September when the first frosts of the coming winter are experienced. They are very troublesome in heavy bracken and heather, in hayfields, especially when hay is being cut, and they are also plentiful around byres.

'Midges are usually most troublesome in the early morning and after sunset, but, especially when it is calm and warm, attacks may occur at any time of the day. They bite very severely during slight drizzling rain. Wind disperses them, but if one is working close to the ground, *e.g.* in forest nursery operations, they may bite even in a wind. Attacks may occur from sunset right throughout the night until 7 or 8 o'clock in the morning. Our records show that they bite as late as 1.30 a.m. (July 30) and 3 a.m. (August 3).' Cameron *et al.* (1946) have made further observations on the biting habits of *C. impunctatus*, and their records show that it is responsible for 90 per cent. of all biting records in the Glasgow area, almost 100 per cent. in the Dundee area, and 70 per cent. in the Edinburgh area, indicating that, midge for midge, this species bites man more frequently than the other species in their catches. They also note a very abnormal sex ratio for this species in the majority of their catches, females being very much in excess of males.

(b) *C. obsoletus* is probably one of the most widely distributed species of *Culicoides* and has been described several times as a new species from different parts of the world.

The following are regarded as synonymous (Edwards, 1939):



- Ceratopogon obsoletus* Meigen, 1818, *Syst. Besch.*, 1, 76.  
*Ceratopogon varius* Winnertz, 1852, *Linn. Ent.*, 6, 35.  
*Ceratopogon sanguisugus* Coquillett, 1901, *Proc. U.S. Nat. Mus.*, 23, 604.  
*Ceratopogon yesoensis* Matsumura, 1915 (*teste* Tokunaga, 1937).  
*Culicoides sanguisugus* Malloch, 1915, *Bull. Illinois Lab.*, 10, 301.  
*Culicoides lacteinervis* Kieffer, 1919, *Ann. Mus. Nat. Hung.*, 17, 47.  
*Culicoides clavatus* Kieffer, 1924, *Bull. Soc. Hist. Nat. Moselle*, 29, 56.  
*Culicoides rivicola* Kieffer, 1924, *Bull. Soc. Hist. Nat. Moselle*, 29, 56.  
*Culicoides heteroceris* Kieffer, 1921, *Bull. Soc. Hist. Nat. Moselle*, 29, 57.  
*Culicoides pegobius* Kieffer, 1922, *Ann. Soc. Sci. Brux.*, 41, 235.  
*Culicoides concitus* Kieffer, 1923, *Ann. Soc. Sci. Brux.*, 42, 71.  
*Culicoides obsoletus* Root and Hoffman, 1937, *Amer. J. Hyg.*, 25, 155.

The male hypopygium is described and figured by Edwards (1939) as well as by Root and Hoffman (1937), while Jobling (1928) describes the structure of the head and mouth-parts.

The species is widely distributed throughout Europe. Kieffer (1925) records it from France, Germany, Austria, Holland and England, and Goetghebuer (1920, 1933) from Belgium. According to Hoffman (1925) and Root and Hoffman (1937) it is also widely distributed throughout the New World, where it has been recorded from British Columbia, Maine, New Hampshire, New York, Pennsylvania, Maryland, D.C., South Carolina, Illinois, Quebec, Tennessee, Kansas, and Montana. Malloch (1915) records the species (as *C. sanguisugus* Coq.) from Illinois, Coquillett (1901) from Maryland, Pratt (1907) from Maryland and British Columbia, and James (1943) from Northern Colorado. Twinn (1931) records *C. obsoletus* from Quebec and Ontario, and Curtis (1941) from Saskatchewan, Alberta and British Columbia.

Tokunaga (1937) records *C. obsoletus* from Sakhalin and Hokkaido, and Kôno and Takahasi (1940) also record it from the Kuriles. In August, 1940, a single female was collected from Manchuria (Tokunaga, 1941). In addition, Sebess v. Zilah (1936) found the species in the Lake Balaton district, and there is a male specimen labelled *C. lacteinervis* in the British Museum from Ludd in Palestine.

*C. obsoletus* is widely distributed throughout Great Britain, the British Museum collection including specimens from Dreghorn (Ayrshire), Loch Assynt (Sutherland), Brodick (Arran), Penshaw (Durham), Staveley (Westmorland), Selsdon and New Maldon (Surrey), Buck's Mills (Devon), Broadheath and Adlington (Cheshire), Snailwell (Cambridgeshire), King's Lynn (Norfolk), Wood Walton Fen (Huntingdonshire), Radwell, Baldock, Clothall and Bricket Wood (Hertfordshire), Watford, Northwood, Kingsbury, Harrow and Pinner (Middlesex), Shefford (Bedfordshire), Frant and St. Leonards (Sussex), Porthcawl (Glamorgan), Burnham (Buckinghamshire), Shotover (Oxford), Llangammarch Wells (Brecknock), Goathorn (Dorset) and Snailbeach (Shropshire). Cameron *et al.* (1946) state that *C. obsoletus* is definitely not a Highland species and occurs most frequently south of the Forth and Clyde.

With regard to the incidence of the species throughout the year, Hoffman (1925) and Root and Hoffman (1937) record it from May 13th to October 10th, while Malloch (1915) records it during April, May, October, and up to November 29th.

Goetghebuer (1920) records *C. obsoletus* (as *C. varius*) in April, May and June, while Twinn (1931) also records it as being most abundant in the early summer. Edwards (1939) states that it is most abundant in June, but occurs throughout the summer and may be very numerous again in September and October. James (1943), however, during the summer of 1942, recorded 659 specimens of *C. obsoletus* caught between May 22nd

and September 18th in a light-trap in Northern Colorado. The species was scarce until July 1st and attained a definite peak of abundance in the late summer. The dates of capture of British specimens preserved in the British Museum range from April 20th to October 21st. Cameron *et al.* (1946) state that the species is found chiefly in the latter half of the summer and extends well into October.

From the Continent *C. obsoletus* has been recorded as breeding in mouldy soil as well as in running water (Thienemann, 1926, 1928; Mayer, 1934*a*); and in Great Britain it has been found in decaying vegetation (Kearns, 1942), in rather dry decaying fungi, in damp debris from a tree-hole, and in sheep dung in fields (Edwards, 1939; British Museum records). Goetghebuer (1936) records the larvae as occurring in water with a pH of more than 7.0, while Mayer (1934*b*) notes that the content of the alimentary canal of the larvae consisted of sand, algae and fine detritus. Cameron and his colleagues (1946) found *C. obsoletus* in both woodland and open country, with a 'strong preference for pasture or cultivated ground.' They also mention a very abnormal sex ratio, only 1 per cent. of their catches being males.

There is very little information in the literature regarding the habits of this species. It has been recorded several times as attacking man (Twinn, 1931; Kearns, 1942; Goetghebuer, 1919, 1923). In addition, Malloch (1915) records it as attacking a horse, and states that it hides in evergreens during the day.

(c) *C. odibilis* was first described by Austen (1921) from a male specimen caught in Palestine. Edwards (1939) has figured the male genitalia and the wing of the female.

The species has a wide distribution throughout Great Britain, and the British Museum collection contains specimens from Letchworth and Radwell (Hertfordshire), Cambridge, Broadheath (Cheshire), Skirwith (Cumberland), and Dingwall (Cromarty), with their dates of capture ranging from May to August. Edwards reared the species from pond-water, while Goetghebuer (1936) records it from stagnant water with an alkaline reaction.

(d) *C. pallidicornis* was originally described from Hungary by Kieffer (1919), while a further illustrated account of the male genitalia was given by Edwards in 1939. *C. disticus* Kieff. and *C. dileucus* Kieff., regarded by Edwards (1939) as synonyms of *C. pallidicornis*, have been described from southern Sweden (Kieffer, 1922) and from Charente-Inférieure (Kieffer, 1921) respectively.

The British Museum specimens are from Corriegills (Arran), Dingwall (Cromarty), Skirwith (Cumberland), Windermere (Westmorland), Snailbeach and Church Stretton (Shropshire), Letchworth, Harpenden and Hitchin (Hertfordshire), Wood Walton Fen (Huntingdonshire), Matley Bog (New Forest), Brasenose (Oxford), Dartmouth and Tipton St. John (South Devon), and Porthcawl (South Wales). Their dates of capture range from May 29th to August.

(e) *C. cubitalis*. Kieffer (1925) first recorded *C. subfascipennis* var.  *analis* Kieff., which Edwards regards as synonymous with *C. cubitalis*, from Belgium, but further records of the distribution of this species outside Britain have not been traced. The sex of the specimen described by Kieffer was not determined, but Edwards (1939) describes the male from British specimens.

The British Museum specimens are from Letchworth and Radwell (Hertfordshire), Dingwall (Cromarty), Catacol and Pirnmill (Arran) and Crag Lough (Northumberland). Their dates of capture range from May 29th to August.

(f) *C. chiopterus* was first described in 1818 (Meigen, 1818). Later Winnertz (1852) described it as a new species, *Ceratopogon amoenus* Winn. Root and Hoffman (1937) have given a figure of the male genitalia, which have also been described and figured by Edwards (1939).

Like *C. obsoletus*, *C. chiopterus* appears to have a wide distribution throughout Europe, Kieffer (1925) having recorded it from Belgium, Germany, Austria and England. It also occurs in the United States, where Root and Hoffman (1937) have recorded its occurrence during May in Baltimore and Maryland. The specimens of this species preserved in the British Museum are from Fatfield (Durham), Letchworth and Radwell (Hertfordshire), and Newmarket (Cambridge). The dates of their capture range from May 4th to September 9th, while Goetghebuer (1920) records the species in May, August and September. Cameron and his co-workers (1946) also found it to be fairly common in Scotland and associated with *C. obsoletus*.

Saunders found the larvae of the species in the sap running from the wounds in elm-trees (Edwards, 1939), a habitat which, so far as is known, is not shared by any other species of *Culicoides* in this country, while Goetghebuer (1936) records *C. chiopterus* as breeding in water with an alkaline reaction.

It will be seen from this review of the literature that previous data concerning the species of *Culicoides* studied by us in the Liverpool area is scanty and mainly confined to morphological studies of adult forms.

#### (4) GENERAL SURVEY OF EXISTING KNOWLEDGE CONCERNING THE BREEDING OF *Culicoides* IN THE LABORATORY

The present writer has traced only four previous accounts of the laboratory breeding of *Culicoides*. The authors of these four papers worked with different species and with different techniques, and their efforts met with varying, but never complete, success.

Patel (1921), who worked with *C. oxystoma*, kept gorged females in a dry tube with a piece of dry blotting-paper for from 50 to 60 hours, and then supplied the flies with small pieces of moist blotting-paper. Under these conditions he found that eggs were laid within 2-3 days after the introduction of moisture in at least 50 per cent. of cases. After oviposition he found that the flies bit readily. The eggs were hatched in the tube in which they were laid, provided that it was kept wet, and the incubation-period varied with the temperature. No information is given regarding the rearing of the larvae, although the length of the larval stage is stated.

Atchley and Hull (1936) record oviposition by wild caught specimens of *C. canithorax*, *C. dovei* and *C. melleus* Coq. up to 12 days after being given a meal of human blood, the number of blood-meals apparently having no effect either on the length of life of the females or on the number of eggs produced. The flies were kept in glass lamp-chimneys, one end of which was covered with cheese-cloth and the other placed on moist blotting-paper or on moist marsh soil in a Petri dish. Single eggs and masses up to 30 were observed on the soil surface and on the blotting-paper. The eggs hatched in 4-5 days or more after oviposition, but the resultant larvae died in less than 24 hours if kept in fresh water, or after a few days if kept in salt water. Dove, Hall and Hull (1932) record that larvae of *C. canithorax* and *C. dovei*, which were captured when more than half grown, were kept in the laboratory for periods of 4-6½ months and were carried over the winter, several adults emerging during the spring months. The larvae were kept at cool temperatures,

supplied with decaying grass-roots and humus, and given brackish water twice a week; the cool temperatures appeared favourable to the larvae, for from those kept at temperatures between 70° and 90° F. not a single adult was reared. These authors also noted that bacterial growth was detrimental to the survival of the larvae.

Steward (1933) observed the complete development of *C. nubeculosus* from egg to adult. He kept wild caught flies in a wooden cage with fine wire gauze at one end, glass in front and a door behind. In the roof of the cage was a large hole, stopped, when not in use, with a wooden plug. A bottle of water containing privet twigs, and plugged with damp wool, was used as a resting-place for the flies. Watered apple, a Petri dish of horse manure and soil, and damp blotting-paper were also provided inside the cage. The interior was darkened by a cloth and blood-meals were given on the hands at frequent intervals. Eggs were laid on the horse manure and the damp wool, and the resultant larvae were kept in tap-water which 'became green' of its own accord.

Jobling (private communication) used the following technique in his breeding of *C. vexans*. Fertilized females were introduced into lamp-glasses standing on Petri dishes lined with a heavy loam, which was kept very moist so that the surface of the lowest part was always covered with a very thin layer of water; these breeding-pots were kept at a temperature of between 14.5° and 17.5° C. Jobling observed that the larvae lived mainly between the very moist lower part and the drier elevated part of the loam, that pupation took place on top of the elevated part, and that the first flies to emerge were all males. It seems apparent from these results that none of the workers quoted maintained any species of *Culicoides* in the laboratory through more than one generation.

In addition to the above-mentioned techniques for rearing species of *Culicoides*, other workers have evolved methods for keeping midges alive in the laboratory for varying periods. Sharp (1928) kept his experimental flies alive for two weeks in a cage which consisted of a silk pyjama-leg stretched over a wire frame, the lower end of the leg being sewn up, while the upper end was closed with a purse-string and could be opened to introduce the arm of the feeder. The closed end was covered with moist cotton wool to give humidity and was wrapped in a black cloth. A piece of cotton wool soaked in honey-water was attached to the dark end of the frame, and a small vessel containing muddy water with algae was suspended from a transverse wire. Oviposition by *C. austeni* was frequently witnessed by Sharp, the numbers of eggs obtained from individual flies varying from 92 to 141. The ova hatched in 40-100 hours after being laid, but the larvae were not seen alive after the fifth day. Buckley (1934), after discarding various techniques, kept his flies alive by two methods: the first was similar to that of Sharp (1928), but in the second he used unglazed earthenware flower-pots, standing on plates of the same material, filled with water; the mouth of each pot was covered with muslin with a sleeve in the centre, through which a test-tube could be inserted and tied vertically, mouth downwards, about one inch from the bottom of the pot; two to four flies were kept in each tube, the end of which was covered with muslin. Both methods Buckley found relatively successful, and his flies survived for up to two weeks. While working on the transmission of *O. gibsoni* by *Culicoides*, Buckley (1938) used a third method, by means of which *Culicoides* survived for periods of up to three weeks in large lamp-chimneys with gauze ends, kept in the darkness of an incubator at room-temperature. After their initial feed on cattle, Buckley found it unnecessary to give a second blood-meal, since the flies survived on raisins which were cut in half and stuck on to the muslin of the chimneys.

Flies which had fed on raisins the first day after hatching were given water on the second, and on the third day an attempt was made to feed them on cattle. These flies were placed in lamp-chimneys with bolting-silk ends, condensation being prevented by dehydrating crystals at the distal mouth of the chimneys. The chimneys were then applied to the shaved flank of a cow for an hour, and it was found that some 50 per cent. of the flies fed within that time. Du Toit (1944) tried to keep *Culicoides* alive by storing them in wooden cages, 5 in. square, which were covered with organdie and provided at one side with a glass panel for purposes of observation. The cages were kept in a room where the relative humidity was maintained at 80 per cent. and the temperature at 78–79° F. Food in the form of 10 per cent. sugar solution, soaked raisins and slices of apple was offered and appeared to be readily taken by the insects, while water was provided by soaking in water small balls of cotton wool covered with muslin which were suspended from the roof of the cages. This technique appears to have proved unsatisfactory, since Du Toit reports a high mortality, principally due to the insects adhering to the moist surfaces in the cages.

It will be seen from this review of the literature that, whereas several workers have reared various species of *Culicoides* in the laboratory from the egg to adult stage, and others have evolved methods of keeping the adult flies alive for varying periods, none have maintained any species of *Culicoides* in the laboratory through more than one generation.

### III. THE SCOPE OF THE PRESENT INVESTIGATION

The foregoing survey of the literature shows that, whereas a considerable number of isolated observations have been made both on the morphology and on the bionomics of certain species of *Culicoides*, none of these observations has resulted in a complete account of the life-cycle and habits of any British species. This lack of knowledge is particularly unfortunate in the case of *C. impunctatus* and *C. obsoletus*, since they are the most wide-spread and annoying species in this country. It was towards the elucidation of the life-history and habits of these species that attention was principally directed during the present investigation; observations were made both in the laboratory and in the field, as it was realized that only in this way could a thorough knowledge of the flies be acquired.

On the laboratory side, the prime object was to evolve a satisfactory method of rearing these species in the laboratory in order to maintain a strain for experimental and class purposes. The technique which was successfully employed for the rearing of these species from the egg to the adult, and for feeding and obtaining eggs and larvae from laboratory-bred flies, will be described later, but it may be stated here that all attempts to maintain the strains to a second generation were unsuccessful.

In the field, advantage was taken of the special facilities offered at Knowsley Park, Liverpool, to study the species (*C. impunctatus* and *C. obsoletus*) under natural conditions. The length of the life-history was studied directly by breeding under field conditions, while a systematic investigation was made of the incidence of the adults and immature stages throughout the year, which gave valuable information confirming and supplementing the direct observations on the life-history made in the laboratory and in the field.

Similar, although less complete, records were also obtained of the life-cycle and habits of *C. odibilis*, *C. pallidicornis*, *C. cubitalis* and *C. chiopterus*.

#### IV. MATERIALS AND METHODS USED IN THE PRESENT INVESTIGATION

##### (1) A DESCRIPTION OF THE SITES FROM WHICH COLLECTIONS OF MATURE AND IMMATURE STAGES OF *Culicoides* WERE MADE THROUGHOUT THE INVESTIGATION

The density of the adults of certain species of *Culicoides* at different seasons of the year was estimated by catching the flies attracted to a black cloth, the observations being made at intervals of a few days during a period extending from April to November, 1945. The site selected for the investigation was an area of land situated close to a lake known as White Man's Dam, on Lord Derby's private estate, Knowsley Park, Liverpool. This area of land was about 300 ft. long by 60 ft. broad, and was densely wooded with *Pinus sylvestris* L., *Rhododendron ponticum* L. and *Betula alba* L., shading a thick undergrowth of *Pteris aquilina* L.

In order to obtain accurate records concerning the number and density of the immature forms of *Culicoides* during a complete year, an area of land which was known to harbour many larvae was selected. The area chosen was a narrow strip of ground about 30 ft. east of the edge of White Man's Dam. On this area were marked out two plots of ground subsequently referred to as 'A' and 'B' (fig. 1), 'A' measuring 220 ft. long by 6 ft. broad, and 'B' 100 ft. long by 12 ft. broad. Two sampling-lines were established on the plots, and from these samples were taken at regular intervals, in the case of plot 'A' from March, 1945, to March, 1946, and in the case of plot 'B' from April, 1945, to April, 1946.

Plot 'A' (Plate III, figs. 1 and 2) had a peaty soil and was quite firm during the summer months and sufficiently firm to walk upon comfortably throughout most of the winter months. The flora of the site was as follows: *Hydrocotyle vulgaris* L., *Rhododendron ponticum* L., *Juncus articulatus* L., *Carex Goodenowii* Gay., *Pteris aquilina* L., *Polytrichum commune* L. (by far the most common plant) and *Gymnocola inflata* Dum.

Plot 'B' (Plate IV, figs. 1 and 2) had a more clayey soil than plot 'A' and was more marshy, owing to drainage from the wood. During no season could the area be walked upon. The flora of this site was as follows: *Hydrocotyle vulgaris* L., *Rhododendron ponticum* L. and *Juncus effusus* L.

The area between plots 'A' and 'B' and the lake edge had a peaty soil and was very wet throughout the year. The flora of this site was as follows: *Cardamine pratensis* L., *Lotus corniculatus* L., *Hydrocotyle vulgaris* L., *Alnus rotundifolia* Mill., *Salix fragilis* L., *Iris pseudacorus* L., *Juncus effusus* L., *Equisetum limosum* L., *Sphagnum subsecundum* Nees. and *Fontinalis antipyretica* L.

On the eastern side the two plots were bounded and shaded—at least during some part of the day—by a small fenced wood sloping gradually down to the marshy land at the lake edge. The flora of this wood was as follows: ground flora—*Scilla noscripta* L. and H. and *Pteris aquilina* L.; shrubs—*Fagus sylvatica* L., *Azalia* sp. and *Rhododendron ponticum* L.; trees—*Acer pseudo-platanus* L., *Aesculus hippocastanum* L., *Betula alba* L. and *Pinus sylvestris* L.

The area to the north of this wooded region was open land, mainly covered with *Pteris aquilina*, with no large trees and only scattered bushes, except for the shore and a small island, situated just off the mainland, where the vegetation was densely bushy but interspersed with a few trees. The area to the east was that used for estimating the adult density and has already been described.

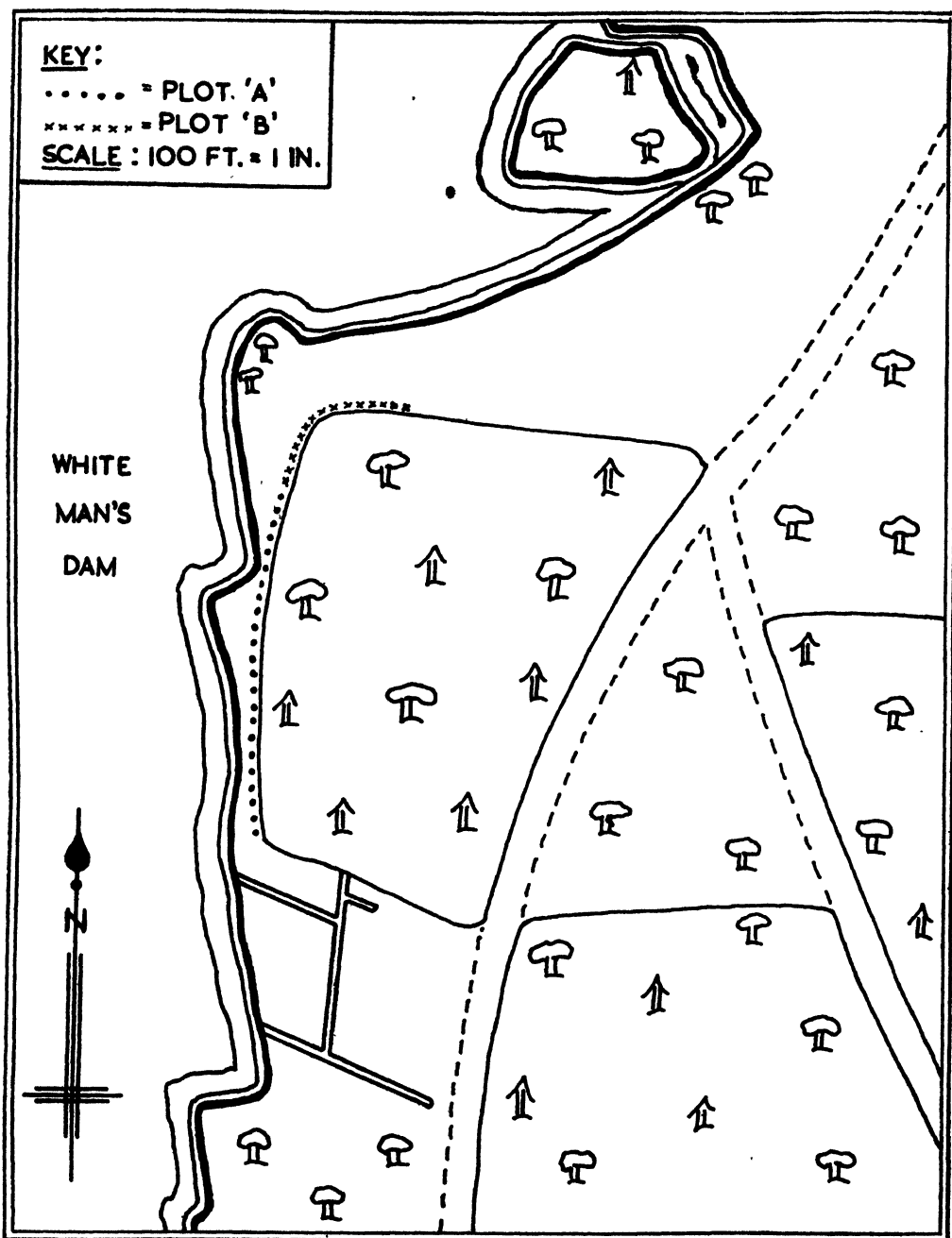


FIG. 1. The site, to the east of White Man's Dam, Knowsley Park, Liverpool, from which the immature stages and adults of *Culicoides* spp. were collected during 1945 and 1946.

The region to the south was crossed at intervals by ditches, with sandstone-block walls (Plate III, fig. 3), draining into White Man's Dam. The flora of these ditches was



FIG. 1. Plot 'A,' on the eastern shore of White Man's Dam, Knowsley Park, Liverpool, showing the location of concentrations of larvae of *C. impunctatus*.

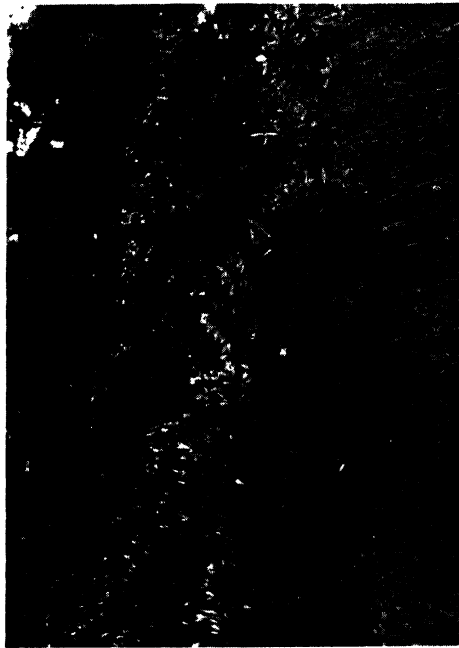


FIG. 2. Detail from fig. 1, showing the breeding-site of *C. impunctatus*.



FIG. 3. Drainage ditches with sandstone-block walls covered with *Peltia epiphylla*, amongst the roots of which, in heavy soil.





FIG. 1. Plot 'B,' on the eastern shore of White Man's Dam, Knowsley Park, Liverpool, showing the location of concentrations of larvae of *C. obsoletus*, *C. odibilis*, *C. pallidicornis* and *C. cubitalis*.



FIG. 2. Detail from fig. 1, showing the breeding-site of *C. obsoletus*, *C. odibilis*, *C. pallidicornis* and *C. cubitalis*.

as follows: *Sphagnum subsecundum* Nees., *Mnium hornum* L., *Mnium* sp., *Dicranella heteromalla* Schp., *Pellia epiphylla* Corda., *Cephaloxia bicuspidata* Dum. and *Calypogeia trichomanis* Corda.

The vegetation over the whole area described was indicative of an acid soil, and various samples, on testing, gave pH values ranging from 5.0 to 6.0.

## (2) METHODS USED FOR THE COLLECTION AND ISOLATION OF IMMATURE STAGES OF *Culicoides*

Throughout the investigation three continuous collections of material were made and examined for the immature stages of *Culicoides*.

The first of these collections, hereafter called collection I, consisted of samples of mud and decaying vegetable-matter from ponds, rot-holes in trees, and damp and shaded spots, which were thought likely to contain larvae and pupae of *Culicoides*, taken from various localities and situations other than plots 'A' and 'B.' The remaining two collections, II and III, were made from plots 'A' and 'B' respectively.

The first collection was made in order to determine the extremes of distribution in relation to different types of conditions under which the midges would breed, rather than series of exact locations of the same type in which they were fairly certain to be breeding. This procedure naturally resulted in the examination of considerable amounts of material which yielded nothing in the way of *Culicoides* larvae or pupae, but which gave information which would be of value in determining the places where it would not be necessary to apply methods of control should these become possible.

The other two collections were made from the two plots 'A' and 'B' which have already been described. From each of these plots six samples of material were collected and examined for larvae and pupae during each two-weekly period throughout the year 1945-46. These samples, each consisting of sufficient material to fill a jar 5 in. deep by 5 in. in diameter, were cut from the surface of the site to a depth of 1 in., previous observations having shown that over 90 per cent. of the larvae occur within the first inch of soil. Each sample therefore represented a surface area of ground of much less than one square foot. They were cut from a sampling-line established on each plot, at intervals of 30 ft. on the line established on plot 'A' and at intervals of 20 ft. on the line established on plot 'B.' Each sample was removed from the area adjacent to the spot where a sample had been removed two weeks earlier.

The pupae of aquatic species of *Culicoides* can sometimes be seen floating on the water surface at the edge of ponds and can be collected by means of a pipette, but only very seldom can larvae be seen swimming over the mud at the bottom of pools, and their collection is difficult owing to their burying themselves in the mud at the slightest disturbance of the water. In general, therefore, it is not possible to discover in the field whether larvae or pupae are present in a given sample of material; this determination has to be made in the laboratory, and the following technique was used for the purpose. Each sample was separately placed in large jars (5 in. in diameter by 5 in. in depth), covered with tap-water and left for a few hours. Occasionally, after the mud had settled, pupae could be seen floating on the surface and larvae swimming round the sides of the jar, particularly round the side facing the light; these were recovered by means of a pipette. The remaining larvae and pupae, if any, in the samples were recovered by the following technique.

Small pieces of the sample were teased out on a wire screen (four meshes to 1 in.). This screen was placed over a second wire screen of a finer mesh (16 meshes to 1 in.), and then the two wire screens were placed over a third screen consisting of a fine bolting-silk (76 meshes to 1 in.). The three screens were placed over an enamel dish, with a blackened bottom, of a similar size (8 in. by 10 in.).

A strong stream of water was then played over the material on the coarse wire screen. Pebbles, plant roots, decaying leaves, moss, etc., were retained by the wire screens, while the mud was washed through on to the bolting-silk. The finest particles were washed through the silk screen, which, however, retained the pupae and larvae of *Culicoides*. The latter were seen wriggling through the sediment left on the screen. After a little practice they could be distinguished from other larvae with the naked eye, their characteristic movement proving of great help in their tentative identification, though the final identification was always checked under a binocular microscope. The larvae and pupae of *Culicoides* which were collected by this means were arbitrarily divided into small (up to 2.5 mm.), half-grown (about 3.5 mm.) and fully grown (over 4.0 mm.).

On several occasions an attempt was made to estimate the accuracy of the laborious technique employed for the recovery of larvae and pupae from their natural medium, and sieved material was once sieved a second time for any immature stages which might have been missed during the first examination. The error was estimated at 0 per cent. for pupae and fully grown larvae, 3 per cent. for half-grown larvae, and up to 20 per cent. for the small larvae. It follows therefore that whenever the presence of small larvae is recorded the total number of larvae as well as the proportion of small forms was in actual fact higher than the figure stated.

Several different species of *Culicoides* emerged from the larvae and pupae collected from numerous samples of material taken from different situations (collection I) and from plot 'B' (collection III). Only one species, however, was bred from plot 'A' (collection II). The very small specific differences in the larvae and pupae make it almost impossible to separate them while alive; consequently it was necessary to recover the larval and pupal pelts of single individuals in order to correlate correctly the immature and adult stages of a species. To this end individual larvae and pupae were set up in small glass phials containing a small amount of the medium in which the larva or pupa was found. This medium was carefully examined beforehand to ensure that it contained no other larvae or old pelts. On many occasions adults were reared and the last larval and pupal pelts recovered, either by teasing out the medium under a dissecting microscope or by floating them out of the medium in water. The larval and pupal pelts were either kept in 70 per cent. alcohol and mounted in pure carbolic acid for examination or mounted directly in polyvinyl alcohol.

### (3) METHODS USED FOR THE COLLECTION OF ADULT *Culicoides*

Four methods were originally used for the collection of *Culicoides* adults: the netting method, the light-trap method, the human-bait method, and a method making use of the attraction of females to black cloth.

By the netting method large numbers of both male and female flies of several species were caught by sweeping vegetation with a large organdie butterfly-net and by sweeping in the immediate neighbourhood of horses, cattle and humans. This method, while it had the advantage of collecting both male and female flies, possessed disadvantages. It

was not easy, under field conditions, to collect the specimens from the net single-handed ; moreover, this method of trapping did not exclude other insects, the *Culicoides* in the catch having to be sorted. In addition, the numbers of flies obtained by netting were extremely variable, and depended too much upon the individual idiosyncrasies of the collector for the method to be used for compiling a record of the incidence of the *Culicoides* sp. caught at regular intervals throughout the year.

The light-trap method of collection also yielded male and female *Culicoides*, but, like the netting method, was not discriminating, and the flies trapped had to be sorted. The variation in the numbers of *Culicoides* caught by this method would be in direct proportion to the numbers in the vicinity of the trap at any particular time, and therefore the method would have proved efficient for making a record of the incidence of *Culicoides* at various times in the year. However, the maintenance of a light-trap in an area without a permanent supply of electricity proved too difficult. Therefore, when sufficient males of the species of *Culicoides* inhabiting Knowsley Park had been collected to enable a correct identification to be made, both this and the netting method of collecting were abandoned.

By the third method, that using human bait, female *Culicoides* were caught in large numbers as they alighted and fed on the exposed parts of the collector. After a few evenings, however, collection by this method became very painful, and it, too, had to be abandoned. But during the course of these preliminary collections it was observed that, when the collector was wearing light-coloured clothing, very few flies would alight on the clothing ; on the other hand, when the collector was wearing dark clothes great numbers of midges would alight on the clothing within the course of a few minutes. Therefore a method was adopted of collecting *Culicoides* as they alighted on a piece of smooth black cloth, 3 sq. ft. in area, hung on bushes at a height of 4-5 ft. This method was decidedly less exacting for the collector, since measures could be taken against the bites of the midges, which, under certain conditions, would attack in hordes. Only female *Culicoides* were attracted to the cloth, and no selection was necessary, since only occasionally did flies of any genus other than *Culicoides* alight.

Howlett (1910) and Eckstein (1920) noticed that mosquitoes were more attracted by dark colours than by light ones ; Brighenti (1930) has carried out some experiments on the attraction exercised by different colours on *Anopheles maculipennis*, and Brett (1938), using *Aedes aegypti*, has carried out experiments with a similar object. But no literature has been traced regarding the attraction of *Culicoides* to dark cloth, and the question needs further investigation.

Collections of *Culicoides* as they alighted on a black cloth 3 sq. ft. in area were made at fairly regular intervals throughout the summer of 1945. As far as possible these catches were carried out on still evenings between two hours and one hour before the official sunset time at Bidston Observatory, and at the same situation in the park throughout the season. This was done in an attempt to standardize variable conditions as much as possible, so that variation in the numbers of midges obtained on different dates would signify actual, as opposed to apparent, variation in the numbers in the field at the time when the collections were made.

The time chosen for the regular collections was not that during which the species of *Culicoides* inhabiting Knowsley Park were most abundant, but of necessity the catches had to be made at this hour. However, there seems no reason to believe that variations

in the numbers of flies of a certain species obtained at this particular time, on different dates, should not represent a variation proportional to that which would have been obtained on the same dates had the catches been made at the peak period of activity of the species under consideration. It must be remembered, however, that the differences in the numbers between one species and another alighting on the cloth may not be significant, since we have no information regarding the relative attractiveness of black cloth for individual species.

During the collection of adult *Culicoides* by any of the four methods described above, a modified form of an aspirator (fig. 2), designed by Du Toit (1944) for collecting *Culicoides* on bait-animals, was used. This apparatus was made up as follows. Pieces of glass tubing 5 in. long, with a bore of approximately 1 in., were prepared by filling one end, to a depth of approximately  $\frac{1}{2}$  in., with freshly mixed plaster of Paris and allowing the plaster to dry. A cork which fitted the tubes was then bored with two holes. Through one hole was passed a piece of glass tubing, 6 in. long and  $\frac{3}{10}$  in. in diameter, bent at an obtuse angle. Through the other hole was fitted a second piece of glass tubing of the same length and bore, but this was bent at an acute angle and the proximal end was covered with bolting-silk to prevent the flies from being sucked into the collector's mouth. The distal end of the second tube was connected to a piece of rubber tubing approximately 18 in. long, and the distal end of this rubber tubing was fitted with a glass mouth-piece. Before use the plaster tubes were moistened by standing in a shallow dish of water.

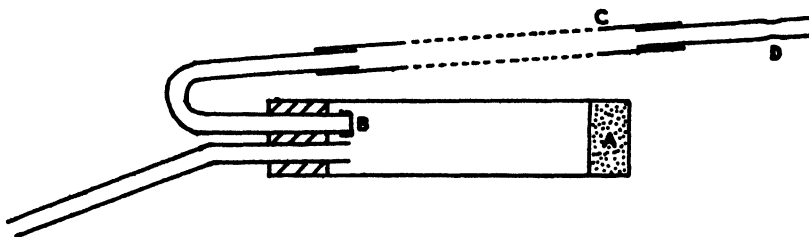


FIG. 2. The aspirator used for the collection of adult *Culicoides* spp. (modified from Du Toit, 1944). A.—Plaster of Paris; B.—Fine gauze; C.—Rubber tubing; D.—Glass mouth-piece.

When about 50 adults had been collected in one plaster-bottomed tube, the plaster end was directed towards the sun and the other end was shaded with the hand; when the flies had collected at the end of the tube directed towards the light, the cork was quickly withdrawn and a cotton-wool plug substituted. This technique proved very satisfactory, and it was only on rare occasions that a fly escaped during the manipulation. The midges captured in the evening were stored in the laboratory for examination later, and it was found that a high humidity was essential for keeping *Culicoides* adults alive even over night. For this purpose the tubes with plaster bases were found to be an excellent container for the transportation and temporary storage of living *Culicoides*, since they permitted any desired degree of moisture and flies survived in them without food for periods of up to five days.

#### (4) METHODS USED FOR THE BREEDING OF *Culicoides* IN THE LABORATORY

##### (a) *Methods of Handling and Feeding Adults*

On the day following their collection in the field all the *Culicoides* were liberated into

a glass lamp-chimney, both ends of which were covered with organdie. A hole wide enough to admit a 1 in. tube was cut in the narrow end and when not in use was always plugged with wool. From this lamp-chimney the flies were withdrawn individually by means of a suction-tube provided with an organdie filter, to prevent the flies from being sucked into the mouth; they were identified, and then each species was distributed to a separate lamp-chimney, the narrow end of which was plugged with wool and the wide end covered with organdie. Live specimens of *C. impunctatus* could always be distinguished with the naked eye, but, whereas *C. obsoletus* could generally be identified by examination with a hand-lens while still in the suction-tube, this method sometimes failed to distinguish it with certainty from *C. chiopterus*. Specimens of doubtful identity were therefore killed with chloroform and examined under a microscope. When all the flies had been identified by these methods the total catch of each species was recorded. All flies belonging to species other than *C. impunctatus* and *C. obsoletus* were then discarded, our attempts to maintain strains of *Culicoides* in the laboratory being confined to these two species only.

As soon as possible after identification the catch of *C. impunctatus* and *C. obsoletus* was offered a blood-meal. At first the flies were fed directly by inverting the lamp-chimneys over the sorter's arm. However, as the season advanced the numbers of flies in each catch became larger, and it became essential to find some other method of feeding the flies. The most satisfactory substitute was found to be the closely shaved (dry shaving was best) ear of a lop-eared rabbit, the rabbit being confined and the ear kept in position by the method commonly used for feeding ticks and described by Jobling (1925). During cold weather it was often found that the temperature of the ears of the rabbit would fall as low as 92° F., at which temperature the midges would not feed nearly so readily as when the temperature of the ears was nearer 100° F. To overcome this difficulty the ear was warmed by placing a warm stage heated to 100° F. between it and the supporting wooden block.

The lamp-chimneys containing *Culicoides* were inverted in turn on the stretched ear of the rabbit, the operation being facilitated by directing the broad end of the chimney towards an electric light, when the flies would collect at this end, and then by quickly withdrawing the cotton plug and placing the narrow end of the chimney on the rabbit's ear. The chimney was kept in position by a clamp and stand and the wide end was covered with a black cloth, which caused the flies to accumulate at the light end near the ear. The midges were found to feed most readily when they had direct access to the closely shaved skin of the ear, although a small percentage could be induced to feed through organdie. Each batch of flies was given approximately half an hour in which to take a blood-meal. The chimney was then removed from the rabbit's ear, again making use of the light tropism, and inverted over a Petri dish lined with moist, but not saturated, filter-paper. The correct degree of dampness is essential: too much moisture results in condensation on the sides of the lamp-chimneys, with consequent trapping of the flies, while too little moisture reduces their span of life. Opportunity to take other blood-meals was given at convenient intervals, usually of two days, each batch having, on an average, four or five such opportunities. Between blood-meals the flies fed readily on balls of cotton wool soaked in 10 per cent. glucose solution or on split raisins placed on the organdie covering the wide ends of the chimneys.

The eggs laid on the moist blotting-paper in the Petri dishes were removed with a camel-hair brush as soon as convenient and placed on the surface of the soil in the breeding-pots.

### (b) *Methods of Rearing Larvae*

The breeding-pots were designed to provide in the laboratory a medium similar to that in which the larvae were found in nature. Earthenware plant-pots, with a diameter of  $4\frac{1}{2}$  in. at their widest part, were soaked in water, and a piece of cotton lint was placed in the inside over the hole in the base. They were then filled to a depth of about 2 in. with the medium in which *C. impunctatus* and *C. obsoletus* were found breeding under natural conditions—in the case of *C. obsoletus* decaying leaves and mud, and in the case of *C. impunctatus* peaty soil—the food-material being arranged to form a slope with a fall of  $1-1\frac{1}{2}$  in. Previously this medium had been dried and heated to about  $80^{\circ}$  C. for approximately half an hour in a hot-air oven, to ensure that any eggs, larvae or pupae of *Culicoides*, together with any predacious arthropods which it might contain, would be killed. In the pots intended for the breeding of *C. obsoletus* no further material was added, but in the pots intended for *C. impunctatus* thoroughly washed plants of *Polytrichum commune* were planted. The prepared pots were covered with lamp-chimneys, with organdie over their narrow ends, and then packed into large enamel trays 2 ft. long by 1 ft. 6 in. broad by 3 in. deep, and surrounded with garden soil to a depth of about 2 in., the water content of this soil being kept at such a level that the breeding-medium in the porous pots was always thoroughly moist and the lowest parts always covered with a thin film of moisture. Known numbers of eggs were then added to the surface of the breeding-medium, usually half way between the lower wetter parts and the more elevated drier parts. Some breeding-pots were kept in the insectarium at a temperature fluctuating between  $22^{\circ}$  and  $24^{\circ}$  C., but the majority were kept at laboratory-temperature, which did not fall below  $16^{\circ}$  C. or rise above  $19^{\circ}$  C. during the observation-period of May, 1945, to May, 1946.

### (5) METHODS USED FOR THE BREEDING OF *Culicoides* UNDER FIELD CONDITIONS

*C. impunctatus* and *C. obsoletus* were maintained in the field in essentially the same way as those kept in the laboratory, the breeding-pots being prepared and the eggs added to them in the laboratory. They were then transported to Knowsley Park, where they were sunk in the ground to a depth of 2 in. at a site where the species to be reared were known to be breeding naturally.

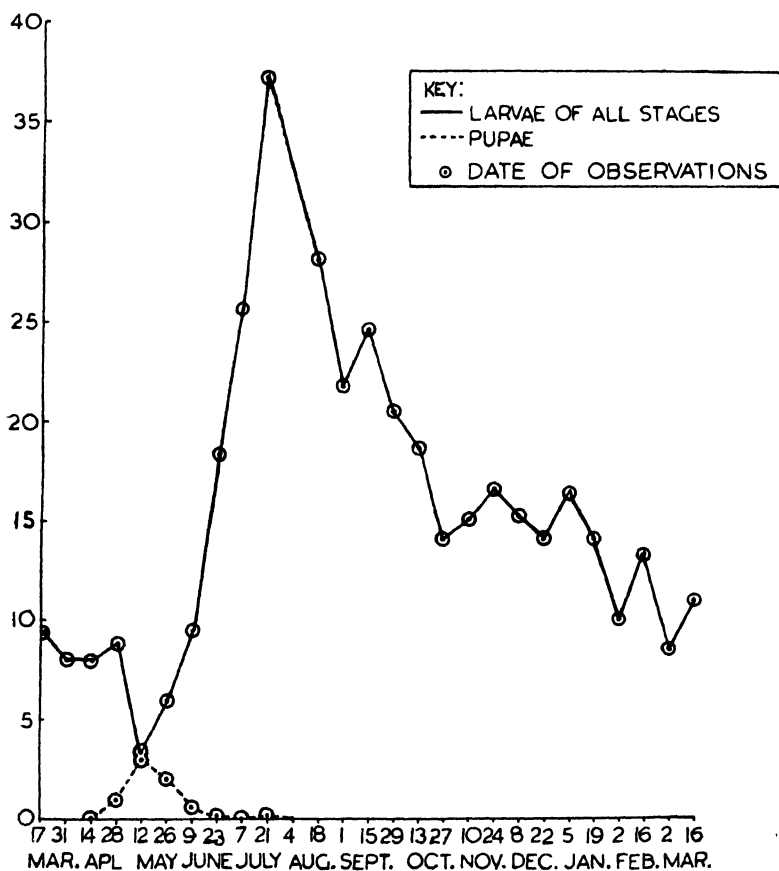
## V. THE RESULTS OBTAINED IN THE PRESENT INVESTIGATION

### (1) *C. impunctatus*

#### (a) *The Incidence of Larvae and Pupae in One Site Throughout a Complete Year*

The only species of *Culicoides* which hatched from pupae recovered from plot 'A' was *C. impunctatus*, and the average numbers of larvae, of all stages, and of pupae obtained from each of six samples cut from the surface of the soil from this plot during each two-weekly period between March, 1945, and March, 1946, are shown in Table I and expressed in graphical form in graph 1 and histogram 1. It is evident from these figures that during March and April, 1945, an average number of nine larvae was recovered from each sample, and that at the beginning of May, 1945, the numbers of larvae recovered had decreased to three per sample. This apparent decrease is, of course, accounted for by the appearance of pupae at that time. The numbers of larvae recovered during the

following period—ending on May 26th—had risen to an average of six per sample, and a steady rise was maintained until July 21st, when the average number of larvae recovered per sample was the maximum recorded throughout the 12 months' observation. An examination of samples taken during the two weeks preceding August 18th showed a decrease in the numbers of larvae. No sampling was possible between July 21st and August 4th. This was unfortunate, since we do not know whether the numbers of larvae recovered during that period would have exceeded the numbers recovered during the



GRAPH 1. The average numbers of larvae, of all stages, and of pupae of *C. impunctatus* recovered from each of six samples taken from plot 'A' every two-weekly period between March, 1945, and March, 1946.

period July 7th–21st. Between August 18th and October 27th there was a steady decrease in the numbers of larvae recovered from the samples. On October 27th the average number recovered from each sample was 14, between that date and March, 1946, the average number of larvae recovered from each sample fluctuated between eight and 16, but at the end of that period the numbers had reached a level comparable with those obtained in March, 1945.

It is of interest to discuss what proportion of small (up to 2.5 mm.), half-grown (about 3.5 mm.) and fully grown (over 4 mm.) larvae occurred in these samples at



different seasons of the year, and to consider the significance of these three groups of larvae, which were well defined to the practised eye. It was surmised that the groups belonged to different instars, but we have traced no information in the literature concerning the number of instars through which the larvae of *Culicoides* pass. Indeed the only information traced concerning the number of larval instars in the Ceratopogonidae is that of Saunders (1924), who states that the larvae of *Forcipomyia* pass through four stages. It has not been possible to determine directly in the laboratory the number of instars of *C. impunctatus* larvae by recovery of successive pelts from individual larvae, and

TABLE  
The average numbers of larvae, of all stages, and the proportions of second, third and fourth instar larvae and of March, 1945,

Two-weekly period ending	No. of samples examined	Total no. of larvae recovered from six samples	Average no. of larvae recovered from each sample	Total no. of 2nd instar larvae recovered from six samples	Percentage of 2nd instar larvae in total recovered from six samples	Average no. of 2nd instar larvae recovered from each sample	Total no. of 3rd instar larvae recovered from six samples
17.3.45	6	56	9.33	0	0	0	0
31.3.45	6	48	8.0	0	0	0	0
14.4.45	6	48	8.0	0	0	0	0
28.4.45	6	53	8.8	0	0	0	0
12.5.45	6	20	3.3	0	0	0	0
26.5.45	6	36	6.0	27	75.0	4.5	0
9.6.45	6	57	9.5	54	94.8	9.0	0
23.6.45	6	110	18.3	107	97.3	17.8	0
7.7.45	6	154	25.6	142	92.2	23.66	11
21.7.45	6	224	37.3	196	87.5	32.66	27
4.8.45	—	—	—	—	—	—	—
18.8.45	6	169	28.1	96	56.8	16.0	73
1.9.45	6	131	21.8	40	30.5	6.66	91
15.9.45	6	148	24.66	27	18.3	4.5	121
29.9.45	6	123	20.5	7	5.7	1.16	116
13.10.45	6	112	18.66	0	0	0	112
27.10.45	6	84	14.0	0	0	0	84
10.11.45	6	90	15.0	0	0	0	80
24.11.45	6	100	16.66	0	0	0	47
8.12.45	6	91	15.1	0	0	0	17
22.12.45	6	84	14.0	0	0	0	8
5.1.46	6	98	16.3	0	0	0	8
19.1.46	6	85	14.1	0	0	0	1
2.2.46	6	60	10.0	0	0	0	0
16.2.46	6	80	13.3	0	0	0	0
2.3.46	6	51	8.5	0	0	0	0
16.3.46	6	66	11.0	0	0	0	0

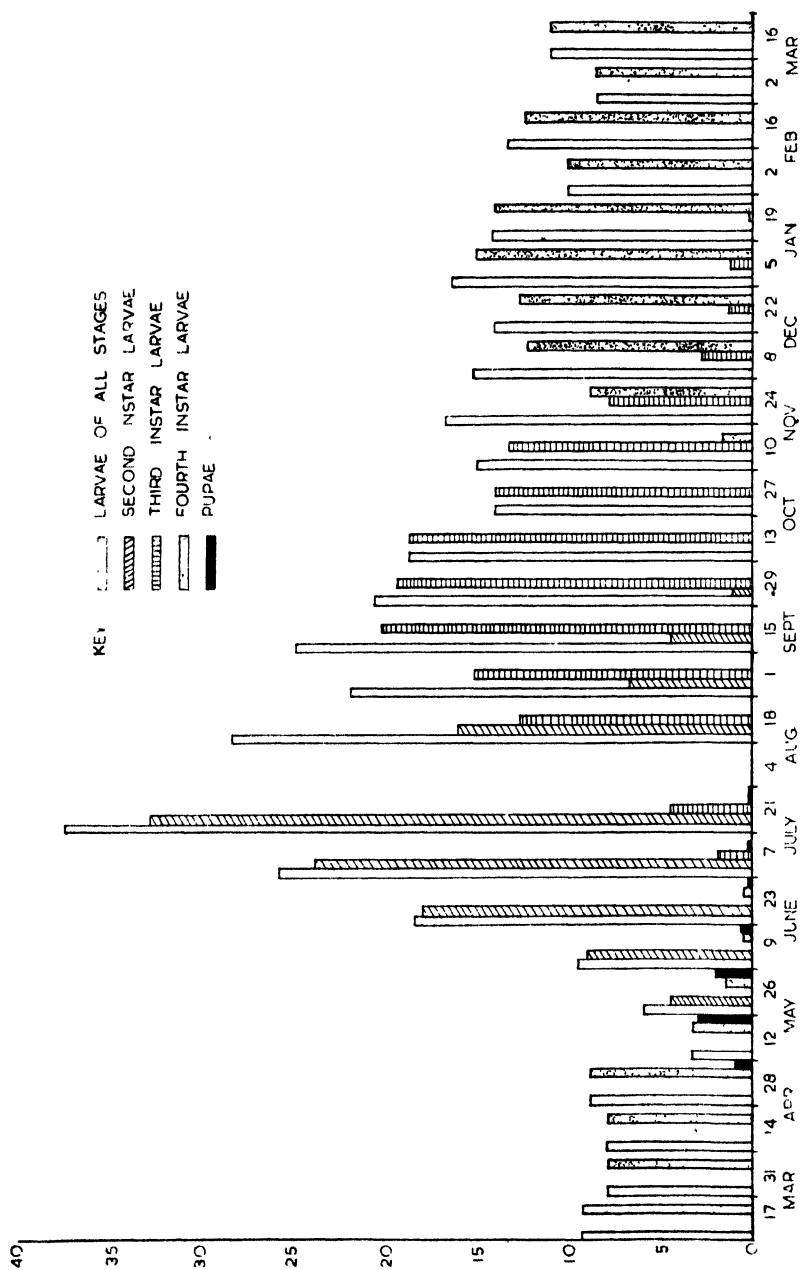
therefore to correlate the three groups with definite instars by comparison with larval pelts of known instars was out of the question. However, it was thought that an indication of the number of instars would be obtained through a series of head-measurements and by applying to these the principles of Dyar's law (Dyar, 1890), by which a skeletal structure, such as the head capsule of the larva, maintains a constant relationship in size in one particular instar and varies uniformly from one instar to the next. The length of the head capsule of 30 larvae from each of the three groups, which we have arbitrarily called small, half-grown and fully grown, from plot 'A' were taken, and without exception these measurements fell into three lots corresponding with the three groups from which the larvae had been taken. Moreover, when each of the three groups of figures was averaged

the following figures were obtained:  $233\mu$  (fully grown),  $176\mu$  (half-grown), and  $133\mu$  (small) for the lengths of the head capsules, and by dividing the smaller figure into the one next above, i.e., 133 into 176, and 176 into 233, the same factor was obtained, i.e., 1.32. This figure represents the ratio of increase as between small, half-grown and fully grown larvae, and, since the factor is the same between small and half-grown and between half-grown and fully grown, it would appear proven, but by the application of Dyar's law only, that these three groups of larvae belong to three separate instars with no other instars interpolated between them. First instar larvae were recovered directly from eggs,

I  
pupae of *C. impunctatus* recovered from each of six samples taken from plot 'A' every two-weekly period between and March, 1946

Percentage of 3rd instar larvae in total recovered from six samples	Average no. of 3rd instar larvae recovered from each sample	Total no. of 4th instar larvae recovered from six samples	Percentage of 4th instar larvae in total recovered from six samples	Average no. of 4th instar larvae recovered from each sample	Total no. of pupae recovered from six samples	Average no. of pupae recovered from each sample
0	0	56	100	9.3	0	0
0	0	48	100	8.0	0	0
0	0	48	100	8.0	0	0
0	0	53	100	8.8	6	1
0	0	20	100	3.3	18	3
0	0	9	25	1.5	12	2
0	0	3	5.2	0.5	4	0.66
0	0	3	2.7	0.5	1	0.16
7.1	1.8	1	0.66	0.16	0	0
12.0	4.5	1	0.44	0.16	1	0.16
43.2	12.6	0	0	0	0	0
69.5	15.16	0	0	0	0	0
81.7	20.16	0	0	0	0	0
94.3	19.33	0	0	0	0	0
100	18.66	0	0	0	0	0
100	14.0	0	0	0	0	0
88.9	13.3	10	11.1	1.66	0	0
47.0	7.8	53	53.0	8.8	0	0
18.7	2.8	74	81.3	12.3	0	0
9.5	1.3	76	90.47	12.66	0	0
8.2	1.3	90	91.8	15.0	0	0
1.2	0.16	84	98.8	14.0	0	0
0	0	60	100	10.0	0	0
0	0	80	100	13.3	0	0
0	0	51	100	8.5	0	0
0	0	66	100	11.0	0	0

and second instar larvae were recovered after the first moult in the laboratory. The average head-length of these second instar larvae was the same as that of the so-called small larvae recovered in the field, i.e.,  $176\mu$ ; therefore these small larvae must have been of the second instar, the half-grown of the third instar, and the fully grown of the fourth instar. Since the so-called fully grown larvae pupated, and since there were no further instars, it seems reasonable to assume that the larvae of *C. impunctatus* pass through four instars; but this assumption is based only on head-measurements and needs careful checking by direct rather than indirect observation. However, throughout the following discussion the small larvae will be called second instar larvae, the half-grown third instar larvae, and the fully grown fourth instar larvae.



The proportions of second, third and fourth instar larvae and pupae occurring in the samples recovered from plot 'A' are shown in Table I and histogram 1. A study of these shows that throughout March and April, 1945, and until the two-week period ending on May 26th, 1945, all the larvae were of the fourth instar and kept at an approximately steady level. During the two-weekly period ending on May 12th the numbers of fourth instar larvae decreased rapidly, and this drop in the incidence of fourth instar larvae continued until July 21st, after which date no larvae of this stage were recovered until the period ending on November 10th. During the intervening period the entire larval population as recorded by us consisted of second and third instar larvae. The complete absence of first instar larvae from our collection-records is probably due to mechanical difficulties of separation, the first stage larvae being less than 1 mm. in length and impossible to recover by the method which we employed. The proportion of second and third stage larvae during this period divides itself into three stages: from May to July the population was composed almost entirely of second stage larvae; during August and most of September the second and third stage larvae were about equally divided; and during the latter half of September and during October the population was almost entirely composed of third stage larvae. The fourth stage larvae began to appear during November, forming some 90 per cent. of the total in December, and from that month onwards until April all the larvae recovered were fully grown. It would appear, then, that *C. impunctatus* hibernates as a fourth stage larva. Saunders (1924) observed that this occurred in the case of *Forcipomyia*, and he states that all known European species of this genus hibernate as third or fourth stage larvae.

The first pupae were recovered during the period ending on April 28th and the last during the period ending on July 21st, the incidence of the first pupae corresponding with the decrease in the numbers of fourth stage larvae. Also, as previously stated, the last fourth stage larvae were recovered during the period ending on July 21st, corresponding with the incidence of the last pupae recovered. The pupal stage is usually of about five days' duration, so that the incidence of the first pupae should correspond (to within a few days) with the incidence of the first adults. During the collection of adults made throughout 1945 the first specimens of *C. impunctatus*, apart from a solitary specimen caught on April 5th, were caught on April 30th.

Between April and September, 1946, continuous sampling similar to that which had been carried out between March, 1945, and March, 1946, was not possible; however, throughout this period, at approximately fortnightly intervals, single samples of soil were taken from plot 'A' and examined for *Culicoides* larvae and pupae. Up to September, 1946, these single samples confirmed the picture of the incidence of larvae and pupae of *C. impunctatus* which had been given during the previous year, although the first pupae and the first second stage larvae were recovered two weeks later.

#### (b) *The Incidence of Adults in One Site Throughout a Complete Year*

Catches of female *C. impunctatus* were made with an aspirator as they alighted on a piece of smooth black cloth 3 sq. ft. in area and hung at a height of 4-5 ft. from the ground. The collections were made at the same place between one and two hours before sunset on still evenings. From March to November, 1945, during 53 such collecting evenings, a total of some 11,900 *Culicoides* adults were captured. The total numbers caught on each

evening and the proportion of the different species making up the total are shown in Table II and graph 2.

A solitary specimen of *C. impunctatus* was caught on April 5th, but no further specimens were captured until April 30th, which date corresponds with the finding of the first pupae of the season during the two-weekly period ending on April 28th. The numbers of this species increased throughout May and reached their peak about the middle of June. Throughout July the numbers of *C. impunctatus* decreased, and the last specimens of the species were caught on August 5th.

A continuous catch throughout the *Culicoides* season of 1946 up to September 30th was not possible, but an effort was made to confirm the results obtained during 1945 by making catches at much less frequent intervals than those made during the previous year. In general the picture given during 1945 was confirmed, but there are one or two discrepancies, possibly due to the wet cool summer of 1946. The incidence of *C. impunctatus* during 1946 agreed with that recorded during 1945, but the first specimens appeared a week later than they appeared in 1945 and the species continued in moderately large numbers throughout August, the last specimens being recorded on August 31st. However, the season of *C. impunctatus* in the neighbourhood of Liverpool was still earlier than that recorded by Cameron and his colleagues in Scotland. This is possibly due to the less rigorous winter experienced in Lancashire.

### (c) *The Life-Cycle as Studied in the Laboratory*

The technique which has been described above for providing female *Culicoides* with an opportunity to take a blood-meal was used with varying success. However, never less than 40 per cent. of one evening's catch of *C. impunctatus* would feed at the first opportunity given to take a blood-meal, and as many as 74 per cent. have been recorded as taking a blood-meal at this time. Subsequent attempts to induce the flies to take a blood-meal resulted in smaller percentages feeding with each successive opportunity given as larger numbers of flies approached the time for oviposition. No flies have been recorded as taking a blood-meal later than two days prior to laying eggs. Individual flies have been recorded as taking up to seven blood-meals, each meal getting successively smaller, until two days prior to egg-laying, and a second blood-meal has been recorded only one hour after the first, when the fly appeared to be fully replete. However, the number of blood-meals taken does not appear to influence the number of eggs laid, similar numbers being laid after one blood-meal as after more than one. The time taken for individuals to become fully gorged varies between three and 20 minutes. Some individuals appear to have difficulty in taking a blood-meal, since they will make several attempts to insert their proboscis, and when this has been accomplished they will remain on the skin with proboscis inserted for periods of up to half an hour, and at the end of that time, on dissection, no trace of blood will be found in the gut. The majority of the flies appeared to have difficulty in withdrawing their proboscis after a meal.

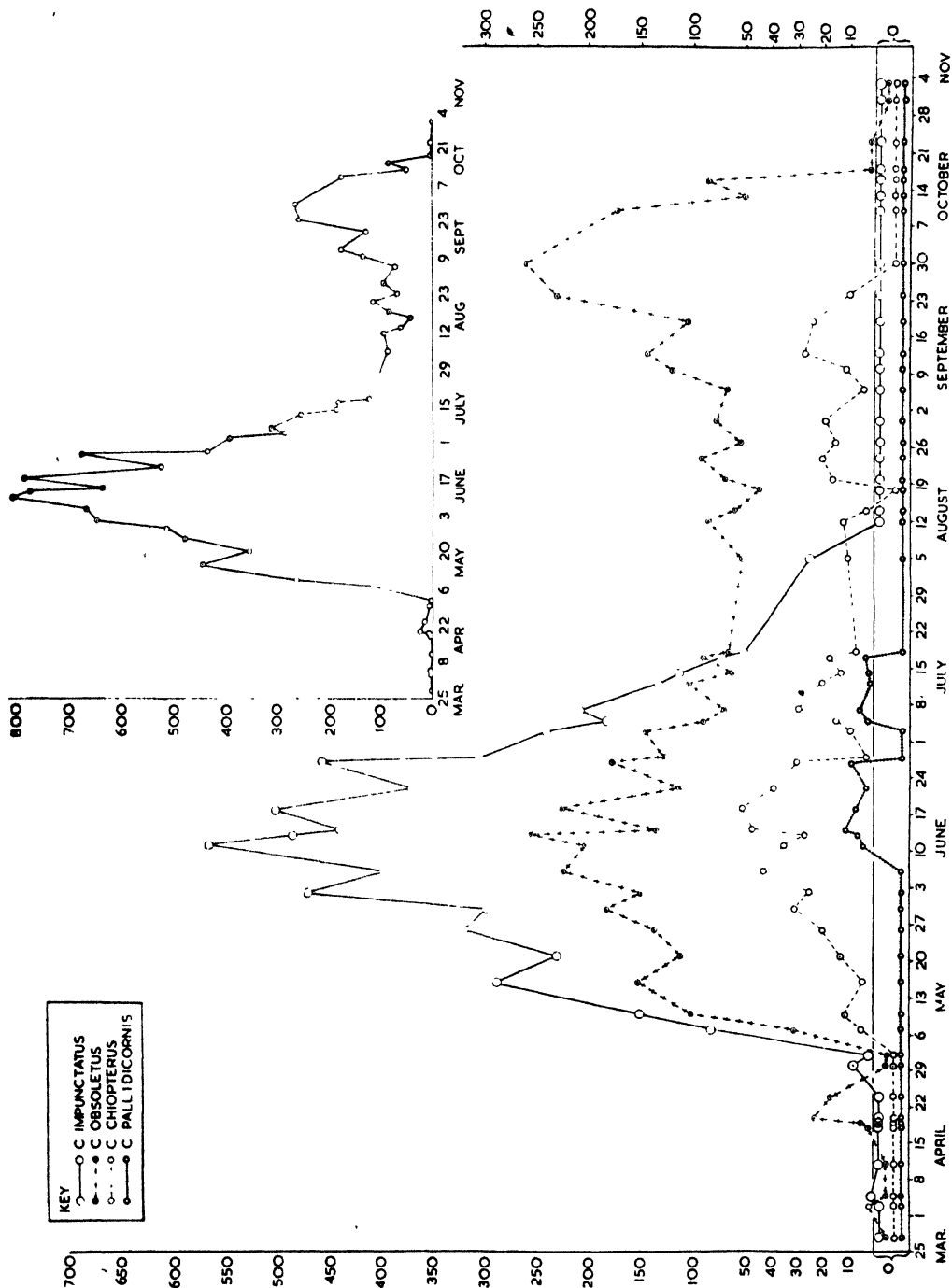
Wild caught flies laid eggs during any time up to 15 days after capture. This would indicate that the normal period taken for development of the eggs is about two weeks, and that eggs laid at shorter intervals were already developing when the parent fly was caught and given its first blood-meal in the laboratory. Females of *C. impunctatus* have been recorded as laying between 30 and 65 eggs in one batch, but about 50 was the usual number deposited, and on no occasion has the author succeeded in obtaining a second

TABLE II

The incidence, throughout 1945, of *Culicoides* spp. adults on one site at Knowsley Park, Liverpool, as shown by catches made as the flies alighted on 3 sq. ft. of black cloth, hung at a height of 4-5 ft., on still evenings between one and two hours before sunset

No. of collection	Date of collection, 1945	Total no. of <i>Culicoides</i> in collection	<i>C. impunctatus</i>		<i>C. obsoletus</i>		<i>C. chiopterus</i>		<i>C. pallidicornis</i>	
			Total	Percent- age of collection	Total	Percent- age of collection	Total	Percent- age of collection	Total	Percent- age of collection
1	28.3	0	0	0	0	0	0	0	0	0
2	3.4	2	0	0	2	100	0	0	0	0
3	5.4	1	1	100	0	0	0	0	0	0
4	11.4	0	0	0	0	0	0	0	0	0
5	18.4	2	0	0	2	100	0	0	0	0
6	19.4	5	0	0	5	100	0	0	0	0
7	20.4	23	0	0	23	100	0	0	0	0
8	24.4	17	0	0	17	100	0	0	0	0
9	30.4	8	8	100	0	0	0	0	0	0
10	2.5	2	2	100	0	0	0	0	0	0
11	7.5	118	82	69.5	31	26.3	5	4.2	0	0
12	10.5	263	150	57.0	102	38.8	11	4.2	0	0
13	16.5	443	287	64.8	152	34.3	4	0.9	0	0
14	21.5	355	230	64.8	112	31.5	13	3.7	0	0
15	26.5	477	320	67.1	137	28.7	20	4.2	0	0
16	30.5	511	298	58.3	182	35.6	31	6.1	0	0
17	2.6	645	470	72.9	150	23.3	25	3.8	0	0
18	6.6	665	397	59.7	225	33.8	43	6.5	0	0
19	11.6	807	566	70.1	202	25.0	35	4.4	4	0.5
20	13.6	773	485	62.7	255	33.0	27	3.5	6	0.8
21	14.6	633	440	69.5	135	21.3	47	7.4	11	1.8
22	18.6	785	502	63.9	225	28.7	51	6.5	7	0.9
23	22.6	524	370	70.6	112	21.4	39	7.4	3	0.6
24	27.6	673	457	67.9	177	26.3	30	4.5	9	1.3
25	28.6	436	306	70.2	127	29.1	3	0.7	0	0
26	3.7	394	240	60.9	145	36.8	9	2.3	0	0
27	5.7	289	182	63.0	90	31.1	15	5.2	2	0.7
28	7.7	311	207	66.6	70	22.5	29	9.3	5	1.6
29	12.7	254	131	51.6	102	40.1	20	7.9	1	0.4
30	14.7	189	112	59.3	62	32.8	13	6.9	2	1.0
31	17.7	183	73	39.9	90	49.2	17	9.3	3	1.6
32	18.7	123	51	41.5	65	52.8	7	5.7	0	0
33	5.8	89	25	28.1	54	60.7	10	11.2	0	0
34	12.8	97	0	0	85	87.6	12	12.4	0	0
35	14.8	63	0	0	60	95.2	3	4.8	0	0
36	18.8	45	0	0	45	100	0	0	0	0
37	20.8	86	0	0	70	81.4	16	18.6	0	0
38	24.8	112	0	0	92	82.1	20	17.9	0	0
39	27.8	70	0	0	55	78.6	15	21.4	0	0
40	31.8	96	0	0	77	80.2	19	19.8	0	0
41	6.9	71	0	0	67	94.4	4	5.6	0	0
42	10.9	131	0	0	120	91.6	11	8.4	0	0
43	13.9	171	0	0	144	84.2	27	15.8	0	0
44	19.9	129	0	0	105	81.4	24	18.6	0	0
45	24.9*	257	0	0	232	90.3	10	3.9	0	0
46	30.9	261	0	0	261	100	0	0	0	0
47	10.10	172	0	0	172	100	0	0	0	0
48	13.10	50	0	0	50	100	0	0	0	0
49	16.10	85	0	0	85	100	0	0	0	0
50	18.10	2	0	0	2	100	0	0	0	0
51	23.10	2	0	0	2	100	0	0	0	0
52	31.10	0	0	0	0	0	0	0	0	0
53	3.11	0	0	0	0	0	0	0	0	0
53	28.3-3.11	11,900	6,392	53.72	4,775	40.12	665	5.59	53	0.44

\* Fifteen female specimens of *C. pulicaris* were caught on this date and are included in the total.



GRAPH 2. The incidence, throughout 1945, of *Culicoides* spp. adults on one site at Knowsley Park, Liverpool, as shown by catches made as the flies alighted on 3 sq. ft. of black cloth, hung at a height of 4-6 ft., on still evenings between one and two hours before sunset. Inset: the total number of *Culicoides* spp. adults caught each evening, as described above, throughout 1945.

batch of eggs from an individual fly. After oviposition, which was usually complete—only on rare occasions were one or two eggs retained—the females as a rule died almost immediately; but, on the only two occasions when the parent females survived, they were given an opportunity to take a blood-meal immediately and they bit viciously. The eggs would emerge like a string of sausages and were usually laid asymmetrically, though sometimes in echelon and sometimes in a double row, the eggs of one row alternating with those of the other, like turned-in footprints. In the laboratory they would be laid on the damp filter-paper, but in nature they have been observed on the surface of the soil in suitable habitats (plot 'A'). Hatching occurred in the laboratory from seven to 20 days after deposition, according to the temperature (16–19° C.), but the average time taken was two weeks. When the first stage larva emerges a small circular cap splits off at the anterior end of the egg, and simultaneously a longitudinal split occurs antero-posteriorly along the dorsal side for approximately a quarter of the length of the egg. This longitudinal split turns sharply to the right or left at its posterior end. These first stage larvae take about two minutes to free themselves from the egg-shell and immediately bury themselves in the breeding-material. Their movement is far more sluggish than that of the larvae of subsequent instars. Subsequent larval stages were located buried with their heads protruding, mainly between the lower and wetter and the more elevated and drier parts of the breeding-medium.

The length of the larval stages of *C. impunctatus* in the laboratory varies considerably, and may last up to seven months. The majority of the larvae pupated within five months, but a proportion from the same batch of eggs always considerably lagged behind in development. The larvae generally pupated in the drier more superficial layers of the breeding-medium, and the pupal stage was usually of five days' duration. The first flies to hatch from a given batch of eggs were all males. However, the number of adults emerging, in relation to the number of eggs transferred to the breeding-pots, was estimated to be as low as 20 per cent. The author believes that the larvae are cannibalistic, which may possibly account to some extent for the low yield.

The above account concerns only larvae kept in the laboratory at temperatures of between 16° and 19° C., not a single fly emerging from the breeding-pots kept in the insectarium at a temperature of 23–24° C. It must be noted here that Dove, Hall and Hull (1932) experienced a similar failure when they attempted to rear the Florida salt-marsh species at temperatures above 70° F.

Flies hatched in the laboratory would copulate in the confinement of a lamp-glass. The act has not been observed by the writer, but living spermatozoa have been found in the spermathecae of laboratory-bred females. Fertilization appears to be independent of a blood-meal, which is usually taken three days after emergence. On two occasions only did females reared in the laboratory survive long enough for complete development of the ovaries. One of these flies laid 45 eggs and the other 56, and both oviposited 14 days after the first blood-meal. These eggs hatched two weeks later, but owing to an accident the strain was not carried further.

#### (d) *The Life-Cycle as Studied in the Field*

*C. impunctatus* was maintained under field conditions by means of a technique similar to that used for rearing the species in the laboratory. Eggs laid on filter-paper in the laboratory were transferred to breeding-pots which were sunk to a depth of about 2 in.



in the soil of a site (plot 'A') in Knowsley Park known to harbour the larvae of *C. impunctatus* naturally.

From a batch of 50 eggs laid on June 5th, 1945, one adult—a male—appeared on August 20th, 1945. This is our only record of a complete life-cycle in one season only. No further hatchings were recorded from this batch until May 26th, 1946. On this date two more males hatched, and during the subsequent six days nine males and 11 females hatched, making a total of 23 flies from a batch of 50 eggs. This is the largest proportion of hatchings that we have obtained from any batch of eggs of this species, although from several batches of eggs laid during the last week in June and during July, 1945, adult flies in smaller numbers were obtained in May and June and in July, 1946, respectively.

#### (e) *Its Habits*

(i) *Hours of Activity.* While the literature indicates that the majority of British species of *Culicoides* are most active during the late afternoon and evening, certain species—*C. nubeculosus* and *C. heliophilus* Edw.—are known to be most active during the middle of the day. Edwards (1921) says of *C. heliophilus* that it had the unusual habit of flying (and biting) chiefly in the hot afternoon sun. By 6 p.m. (summer time) it had disappeared and was being replaced by hosts of *C. impunctatus*.\* Cameron (1946) states of *C. impunctatus* that it is usually most troublesome in the early morning and after sunset, while Steward (1933) found *C. nubeculosus* to be most abundant between the hours of 10.30 a.m. and 1.0 p.m. B.S.T., and states that *C. pulicaris*, *C. obsoletus*, *C. parroti* and *C. stigma* Mg. are caught most readily between 7.0 and 10.0 p.m. B.S.T. Since more precise records of the times of activity of British species have not been traced, an attempt was made to determine the hourly peaks of abundance of the species of *Culicoides* inhabiting Knowsley Park.

Observations were made on different occasions from 11 a.m. G.M.T. until sunset and from dawn until 7.30 a.m. G.M.T., the flies being caught with an aspirator, by the technique already described, as they alighted on a black cloth.

The results of these catches, so far as they concern *C. impunctatus*, are recorded in Table III and graph 3. This species is not much in evidence prior to three hours before sunset, but two hours before sunset it becomes numerous, and its numbers reach a maximum about an hour and a half later (i.e., half an hour before sunset) as recorded by us. Towards sunset its numbers decrease markedly, possibly owing to the drop in temperature or to the fact that a failing light may cause the cloth to become less attractive as the light diminishes. As the method used for catching the flies was not practicable in a failing light, figures have not been obtained of the incidence of the species after half an hour past sunset. However, reports obtained from the inhabitants of certain parts of the Highlands of Scotland indicate that the species is active throughout the night, though to a lesser degree than in the evening. A catch made on June 23rd, 1946, starting just after sunrise, indicates that *C. impunctatus* is also active during the early morning, though far less so than in the evening, and that the midges disappear approximately four hours after sunrise. Throughout the day they are very little in evidence, except in very sheltered and shady places and during sultry thundery weather. Drizzling rain does not deter the activities of *C. impunctatus*, although the slightest breeze does so.

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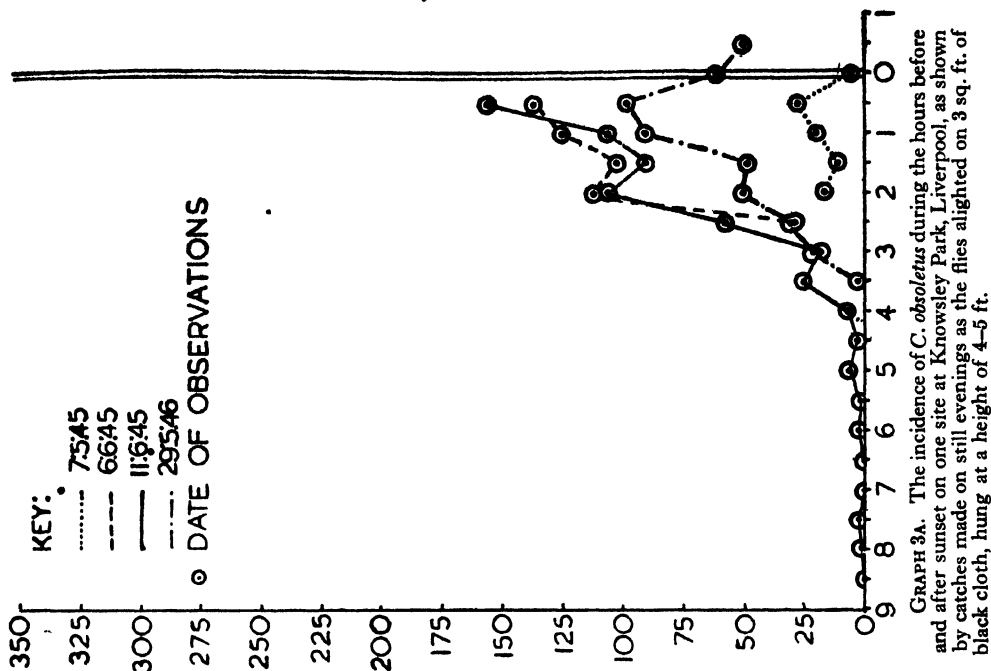
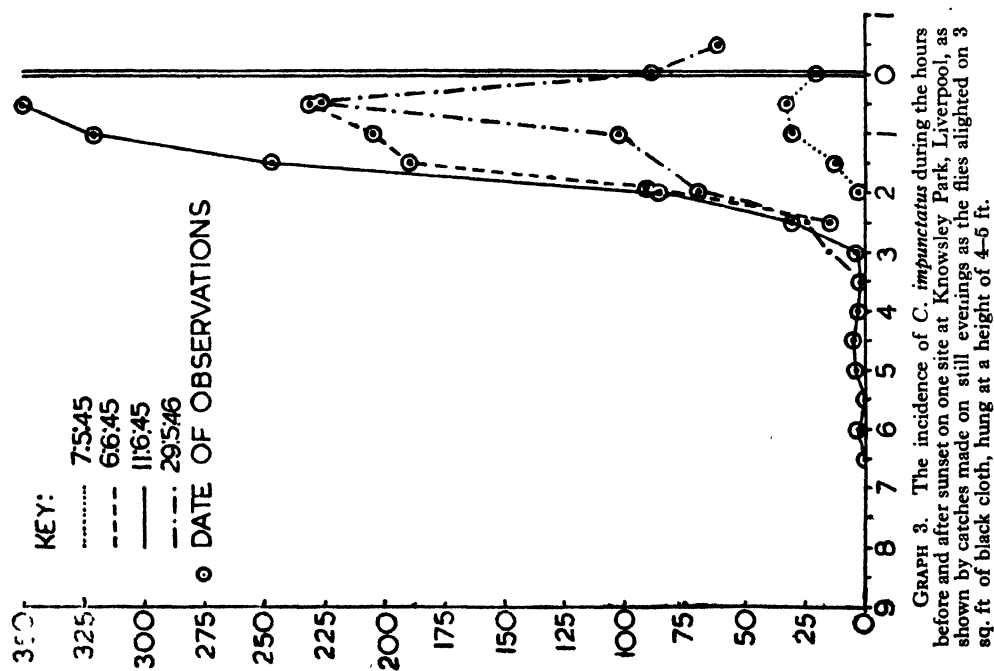
\* Originally referred to by Edwards as *C. arcuatus* but later found to be *C. impunctatus* (Edwards, 1939).

TABLE III

The incidence of *Culicoides* spp. adults on one site at Knowsley Park, Liverpool, as shown by catches made on still days as the flies alighted on 3 sq. ft. of black cloth, hung at a height of 4-5 ft.

Date	Sunset, G.M.T.	Time of collection, G.M.T.	Total no. of <i>Culicoides</i> collected	<i>C.</i> <i>impunctatus</i>	<i>C.</i> <i>obsoletus</i>	<i>C.</i> <i>chipterus</i>	<i>C.</i> <i>pallidicornis</i>
7.5.45	19.51	17.25-17.50	19	2	17	0	0
		17.50-18.20	25	12	11	2	0
		18.20-18.50	53	30	20	3	0
		18.50-19.20	59	32	27	0	0
		19.20-19.50	25	20	5	0	0
6.6.45	20.34	17.30-18.0	45	15	28	2	0
		18.0-18.30	212	91	112	9	0
		18.30-19.0	314	189	102	23	0
		19.0-19.30	533	208	125	20	0
		19.30-20.0	375	230	137	8	0
11.6.45	20.39	11.0-11.30	0	0	0	0	0
		11.30-12.0	0	0	0	0	0
		12.0-12.30	1	0	1	0	0
		12.30-13.0	2	0	2	0	0
		13.0-13.30	0	0	0	0	0
		13.30-14.0	0	0	0	0	0
		14.0-14.30	5	3	2	0	0
		14.30-15.0	1	0	1	0	0
		15.0-15.30	10	4	6	0	0
		15.30-16.0	8	5	3	0	0
		16.0-16.30	15	3	12	0	0
		16.30-17.0	26	1	25	0	0
		17.0-17.30	21	3	18	0	0
		17.30-18.0	94	30	57	7	0
		18.0-18.30	201	85	106	10	0
		18.30-19.0	351	246	90	15	0
		19.0-19.30	456	320	112	20	4
		19.30-20.0	619	350	157	12	0
29.5.46	20.25	16.30-17.0	2	0	2	0	0
		17.0-17.30	25	0	20	5	0
		17.30-18.0	52	15	31	6	0
		18.0-18.30	76	24	50	2	0
		18.30-19.0	125	71	49	5	0
		19.0-19.30	201	101	90	10	0
		19.30-20.0	336	226	98	12	0
		20.0-20.30	150	88	60	2	0
		20.30-21.0	112	62	50	0	0
23.6.46	Sunrise, 3.43	3.55-4.25	30	30	0	0	0
		4.25-4.55	35	35	0	0	0
		4.55-5.25	34	34	0	0	0
		5.25-5.55	37	37	0	0	0
		5.55-6.25	24	24	0	0	0
		6.25-6.55	10	10	0	0	0
		6.55-7.25	12	12	0	0	0
		7.25-7.55	1	1	0	0	0

(ii) *Flight Range*. Hill and Roberts (1947) envisage the control of *Culicoides*—in so far as *C. impunctatus* is concerned—under the experimental conditions which are described. However, control of this species over the very large areas in which they are believed to breed—for instance, the Highlands of Scotland—would probably prove to be an uneconomic proposition. On the other hand, the proposition might not be so uneconomic to local authorities if it could be stated that *Culicoides*, under normal conditions,



does not fly a great distance from its breeding-place, since this would imply that an area once cleared of its population of *Culicoides* larvae would not become reinfested immediately through adults invading from other areas.

The literature which we have traced concerning this subject is scanty, but all the references indicate that *Culicoides* does not normally occur at great distances from its breeding-grounds, unless blown by the prevailing wind.

Steward (private communication) is of the opinion that *C. nubeculosus* does not normally stray more than about 100 yards from farm-yards, while Myers (1934) in the Bahamas found no infestation of *Culicoides* which was not well within half a mile of a favourable breeding-ground. Nevertheless, drift with the wind must be taken into consideration, since Whitehead (1935), during an investigation into damage caused to cattle by an infestation of *C. variipennis* Coq. breeding in a North Canadian river, found that the midge infestation occurred at short distances from the river, but that this distance varied according to the direction of the prevailing wind and to the topography of the land, though in general the infestation extended from the river for an approximate distance of two miles. In addition, Edwards (1939) notes that in 1938, during the course of investigations into the aerial drift of small insects, Hardy obtained several specimens of *C. pulicaris* over the middle of the North Sea, while Glick (1939) collected *Culicoides* at altitudes of from 200 to 13,000 ft. Painter (1926) also collected *C. furens* at a distance of 2,500 ft. from the nearest breeding-place.

Transport by the wind would appear to be passive, since Bequaert (1924) noted that a perfectly calm atmosphere is necessary for the flight of *C. furens*, the slightest breeze driving the insects into hiding—a fact which has been confirmed in our work in so far as *C. impunctatus*, *C. obsoletus* and *C. chiopterus* are concerned.

The observations of the present writer indicate that *C. impunctatus*, under still conditions, does not occur far from a potential breeding-ground. On several occasions catches were made in the open at various distances from a known breeding-site. These collections were made in the usual manner, i.e., with a black cloth hung at a height of 4–5 ft. from the ground, and each was of 10 minutes' duration and made during the peak period of activity of *C. impunctatus*. The results, given in the following table, are those obtained on one evening only, but they are typical of a series of results obtained in a similar manner.

Date of catch	Duration of catch	Distance from potential breeding-ground	No. of <i>C. impunctatus</i> in catch
14.6.45	10 min.	0 yards	72
		50 "	39
		100 "	22
		150 "	10
		200 "	2
		300 "	0

It will be seen that the numbers in each catch decreased as the distance between the breeding-ground and the site of the catch increased, until at a distance of 300 yards from the breeding-ground no specimens of *C. impunctatus* were captured.

(iii) *Host Preferences*. As previously stated, our search of the literature has brought to light very little information regarding the biting habits of *C. impunctatus*. We have

no information on the natural hosts of this species, but we can state that it has a very marked preference for man when compared with the other species of *Culicoides* inhabiting Knowsley Park. Of the catches collected by means of the black cloth *C. impunctatus* averaged some 60 per cent. of the total during the whole of 1945. On the other hand, in various catches made at similar times and under similar conditions, but using the human-bait method for collecting the flies instead of the black cloth, this species comprised an average of over 90 per cent., a fact which would indicate that it had a much greater preference for man than had the other species (*C. obsoletus* and *C. chiopterus*) in the catches. It might be argued that over 90 per cent. of the *Culicoides* females in the field at the time when the catches were made belonged to the species *C. impunctatus*, and that the species in reality had no real preference for man, the smaller percentages of this species in the catches made on the black cloths really being due to the cloths being less attractive to this species. However, when sweeps of the vegetation with an organdie butterfly-net were made between one and two hours before sunset on various occasions, the percentages of the various species of *Culicoides* recovered by this means approximated to those recovered on the black cloths, i.e., 60 per cent. *C. impunctatus*, thus indicating, although not proving, that the species really had a greater preference for man than had either *C. obsoletus* or *C. chiopterus*.

A further point, noted also by Cameron *et al.* (1946), was observed in the catches made by the organdie butterfly-net. The proportions of males and females which hatched from pupae recovered from plot 'A' were approximately the same. But in the catches made with the butterfly-net only 3 per cent. of the total number of *C. impunctatus* caught by this means were males. At present we cannot explain this difference in the sex ratio, unless it is due to the greater preference of females to sheltering in herbage.

The author has only one record of *C. impunctatus* biting inside buildings, and it would appear that it does not normally enter buildings, though it often does so owing to the fact that it is attracted to artificial light.

(iv) *Range of Breeding-Grounds.* Plot 'A,' in which *C. impunctatus* was found breeding in some numbers, has already been described. The outstanding characteristic of all the situations in which the immature stages of this species have been found is peaty soil with an acid reaction. It seems from our observations that the species will not breed in areas which are under water throughout the year, and that, although a soil-breeder, it will not breed in sandy or clay soil.

(f) *The Morphology of the Adult, Together with an Account of the Previously Undescribed Egg, Larva and Pupa*

References to descriptions of the adult females of *C. impunctatus* have already been given, and we have nothing to add to these descriptions. However, it seems desirable to give fuller details of the male genitalia of the species (fig. 3, A) than those given by Edwards (1939).

The tergite has short processes which are almost triangular and slightly convergent, and there is a small median notch. The inner side of the coxite bears a dense, short, stiff pubescence on an inconspicuous hump, and the style is rather short and slightly enlarged at the rounded tip. The ventral root is short. The aedeagus has a distinct anteroventral margin, and the parameres are long and angled near the base and bear a

small tuft of fine hairs at the tip. The sternite has a shallow excavation and the membrane is bare. The cerci are variable in size.

The author has examined the specimens in the British Museum on which Edwards

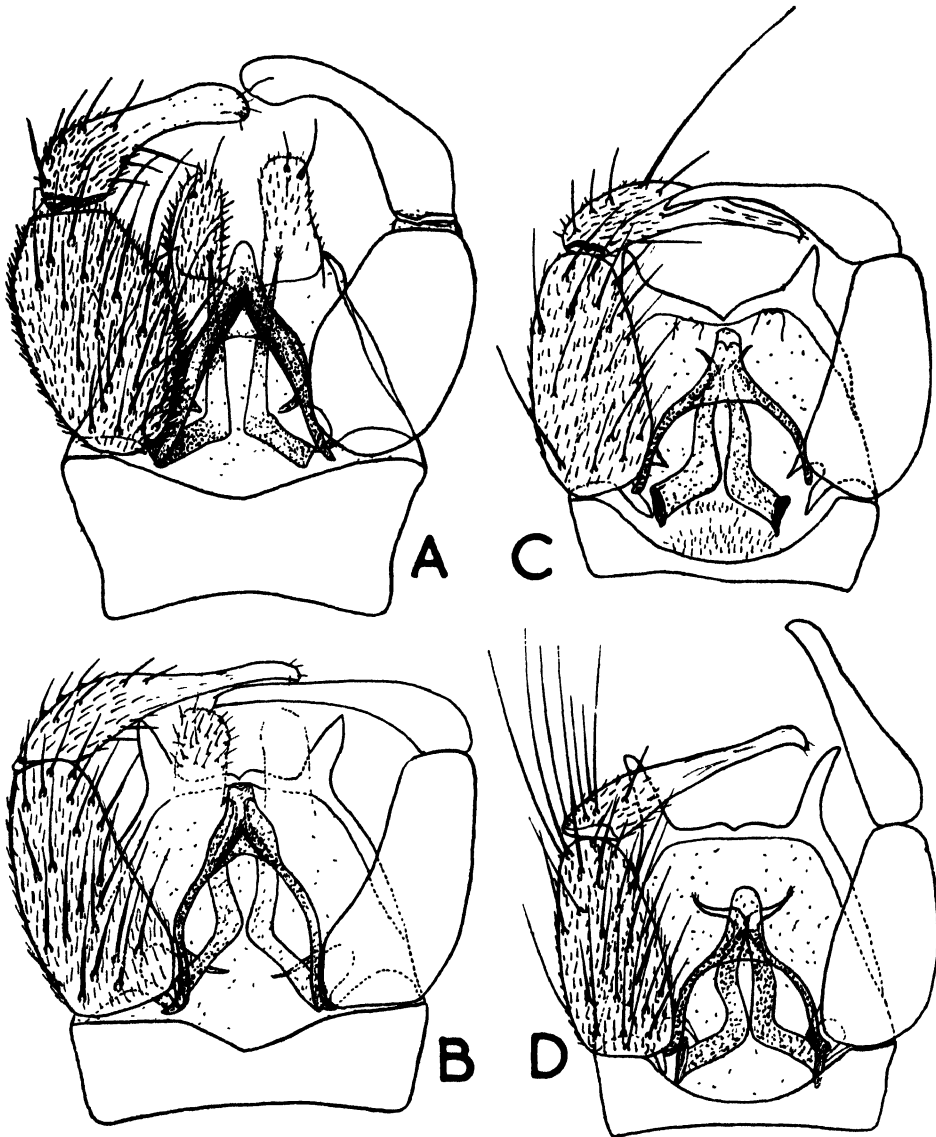


FIG. 3. Ventral view of the male genitalia of *Culicoides* spp. ( $\times 300$ ). A.—*C. impunctatus*; B.—*C. odibilis*; C.—*C. pallidicornis*; D.—*C. cubitalis*.

based his description of the male genitalia of this species. These specimens were mounted without pressure in Canada balsam after staining with carbol fuchsin, while our specimens were mounted while fresh without pressure in polyvinyl alcohol. It is possible that these

two different methods of preparation account for the slight discrepancies between our description and that of Edwards, who draws the parameres with a curved tip and describes the tergite as without a notch. However, Edwards does not mention the anteroventral margin of the aedeagus, and the relative length of the aedeagus and parameres to the length of the tergite in our specimens would appear to be greater than in the British Museum specimens.

The eggs of *C. impunctatus* (figs. 4, A, and 4, B) have an average length of  $490\mu$  and an average breadth of  $80\mu$  one hour after being laid; they are cigar-shaped, but slightly more pointed towards the posterior end. They are light in colour when first laid, and become dark greyish-brown within half an hour. The chorion has rows of sucker-like structures over its whole surface (fig. 4, C). These rows are single dorsally and double ventrally. Patel (1921) noticed similar structures on the eggs of *C. oxystoma* and expresses

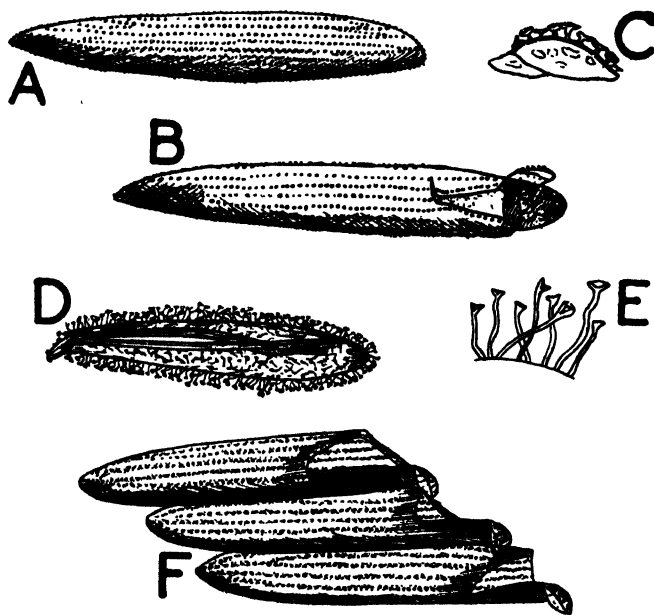


FIG. 4. Eggs of *Culicoides* spp. A.—Lateral view of the unhatched egg of *C. impunctatus* ( $\times 112.5$ ); B.—Dorsal view of the egg of *C. impunctatus*, showing the method of splitting and the first stage larva emerging ( $\times 112.5$ ); C.—The sucker-like structures on the chorion of the egg of *C. impunctatus* ( $\times 525$ ); D.—The chorion of *C. obsoletus*, showing the method of splitting ( $\times 112.5$ ); E.—The sucker-like structures on the chorion of *C. obsoletus* ( $\times 525$ ); F.—Hatched eggs of *C. odibilis*, showing the method of splitting ( $\times 112.5$ ).

the view that possibly they act as floats. The first stage larvae are approximately 0.8 mm. long when first hatched, and have a pale yellow head which is  $76\mu$  long by  $61\mu$  broad.

The larvae of *C. impunctatus*, when mature, have an average length of 5.0 mm. and are semi-transparent, with a dark-brown head which is heavily chitinized (fig. 5). The average length of the head (fig. 7, A) is  $233\mu$ , and it is just over half as broad as it is long. In cross-section it is cylindrical and does not appear to be flattened on its ventral surface. Dorsally, the head capsule is divided into three plates—a median, called 'clypeal' by Carter, Ingram and Macfie (1920), and two lateral or epicranial plates. These are separated by sutures, along which rupture occurs when the larva pupates and the pelt is

cast. Several pairs of bristles are arranged along the sutures. Two pairs of small bristles occur near the posterior margin of the epicranial plates, and two pairs of large bristles are situated anterior to these on the edge of the epicranial plates. Anterior to these is a pair of smaller bristles, also situated on the edge of the epicranial plates. Situated at about a third of the length of the head from the anterior end are two more pairs of large bristles, the anterior pair occurring on the epicranial plates and the posterior pair, which are

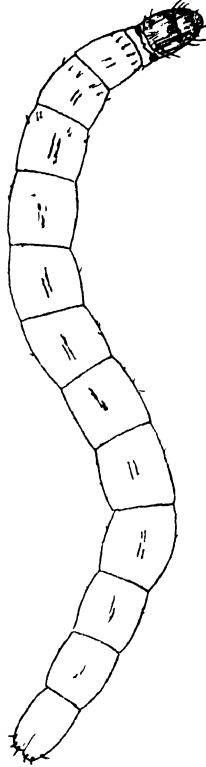


FIG. 5. A fully grown larva of *C. impunctatus*; length 5.0 mm.

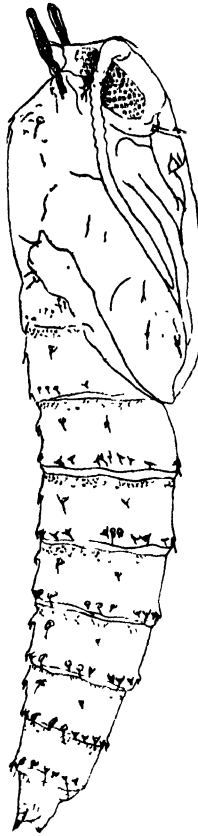


FIG. 6. A female pupa of *C. impunctatus*; length 2.1 mm.

surrounded posteriorly by the eyes, occurring on the clypeal plate. Anteriorly another pair of large bristles occurs on the clypeal plate, and a further pair just anterior to the antennae. Ventrally the head bears three pairs of large bristles, one pair situated approximately half-way along the length of the head and the other two pairs situated anteriorly to this pair. Laterally there are four pairs of large bristles situated as shown in fig. 7, B. Anteriorly one large pair and one small pair of bristles are situated on the labrum. Just behind the maxillae is a further pair of large bristles and posterior to these a further small pair.



The eyes are kidney-shaped and heavily pigmented, and are normally situated anterior of the middle of the head capsule; but as the larva approaches pupation they are retracted backwards and occur near the posterior margin of the head.

The antennae are situated near the anterior extremity of the head and are minute membranous structures with a relatively large lobe, which appears to bear a stoutish spine, and four smaller lobes (fig. 7, D).

The labrum is almost semicircular; it is membranous and projects anteriorly. On its ventral margin it bears two groups of five papillae, a pair of large hairs and a pair of smaller ones (fig. 7, E).

The maxillae are small membranous structures bearing a group of four papillae at their tip, a large papilla bearing a smaller one near its proximal end, and a group of three papillae near its base (fig. 7, C).

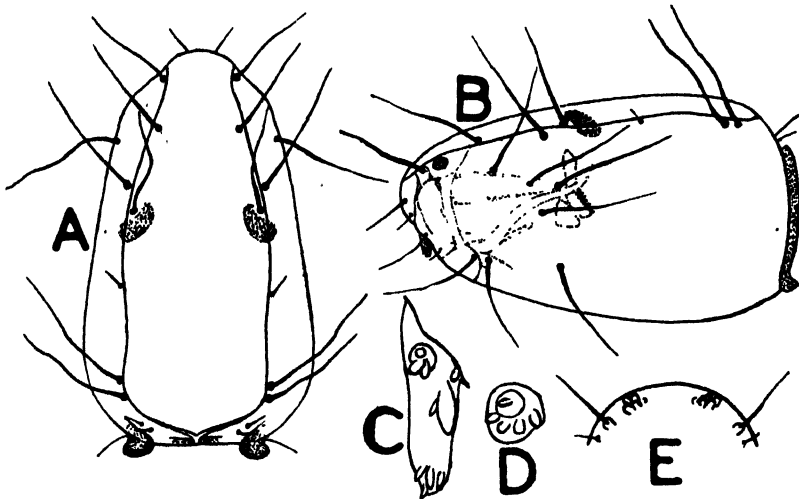


FIG. 7. A fully grown larva of *C. impunctatus*. A.—Dorsal view of the head ( $\times 225$ ); B.—Lateral view of the head ( $\times 225$ ); C.—Maxilla ( $\times 1,050$ ); D.—Antenna ( $\times 1,050$ ); E.—Labium ( $\times 450$ ).

The mandibles are heavily chitinized, pointed and slightly curved, and bear a small tooth about half-way along their length (fig. 8, A).

The labial plate is heavily chitinized, and has a few small indentations, which can hardly be designated teeth, on either side.

It appeared to Carter and his colleagues that the teeth arising from the posterior margin of the hypopharyngeal sclerite afforded an important means for larval differentiation, and the present author is of a similar opinion. The hypopharyngeal sclerite of *C. impunctatus* is rather heavily chitinized, and, seen from the dorsal side, there is a row of some 10 or 12 small pointed teeth on either side. Ventral to this is a second row of five or six larger pointed teeth on either side (fig. 8, A).

The body of the larva of *C. impunctatus* is cylindrical and composed of 12 segments, which are slightly longer than they are broad. The terminal segment is bluntly rounded and bears five pairs of small hairs, while the other segments bear a few delicate hairs which are difficult to see. However, for the species we have examined they do not appear to have any systematic importance.

The first segment immediately behind the head is constricted, giving the body the appearance of being composed of 13 segments. As noted by Carter *et al.*, this neck is entirely devoid of hairs, and the presence of imaginal buds in the three following segments, which swell prior to pupation, indicates that it cannot be the first thoracic segment. There appear to be no retractile gills extending from the last segment of the larva of this species.

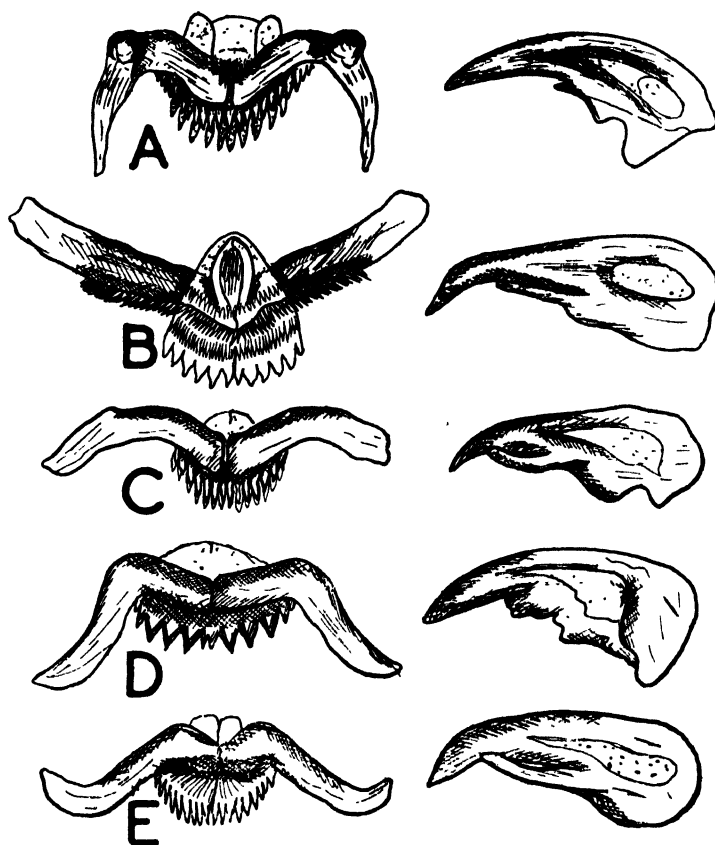


FIG. 8. The hypopharyngeal sclerites and mandibles of *Culicoides* spp. ( $\times 750$ ). A.—*C. impunctatus*; B.—*C. obsoletus*; C.—*C. odibilis*; D.—*C. pallidicornis*; E.—*C. cubitalis*.

The pupa of *C. impunctatus* (fig. 6) is about 2.1 mm. in length. The operculum (fig. 9, A) is covered with dark, short, blunt spines, which tend to be denser in the female than in the male; anteriorly these merge into longer blunt protuberances. The respiratory trumpets (fig. 10, A) are short, straight and raised on a stalk, which is about one quarter of the length of the trumpet. The length of the trumpet is about  $168\mu$  and the length of the stalk about  $40\mu$ . The trumpet, which is pale at its base and tip and darker in the central portion, bears no knob-like processes along its length, and the main tracheal trunk may or may not be divided into two at the base of the trumpet; but there are no lateral branches and the trunk ends in a fan-like group of short, blunt, spiracular openings (usually six).

The anteromarginal tubercle (throughout the description of the tubercles the terminology of Carter *et al.* (1920) will be used) on the cephalothorax is prominent and bluntly conical, and bears a long stout bristle. The anterodorsal tubercle is large and blunt, and bears two long bristles, the outer one slightly longer than the inner. These bristles are more than half as long as the respiratory trumpet. The dorsolateral tubercle

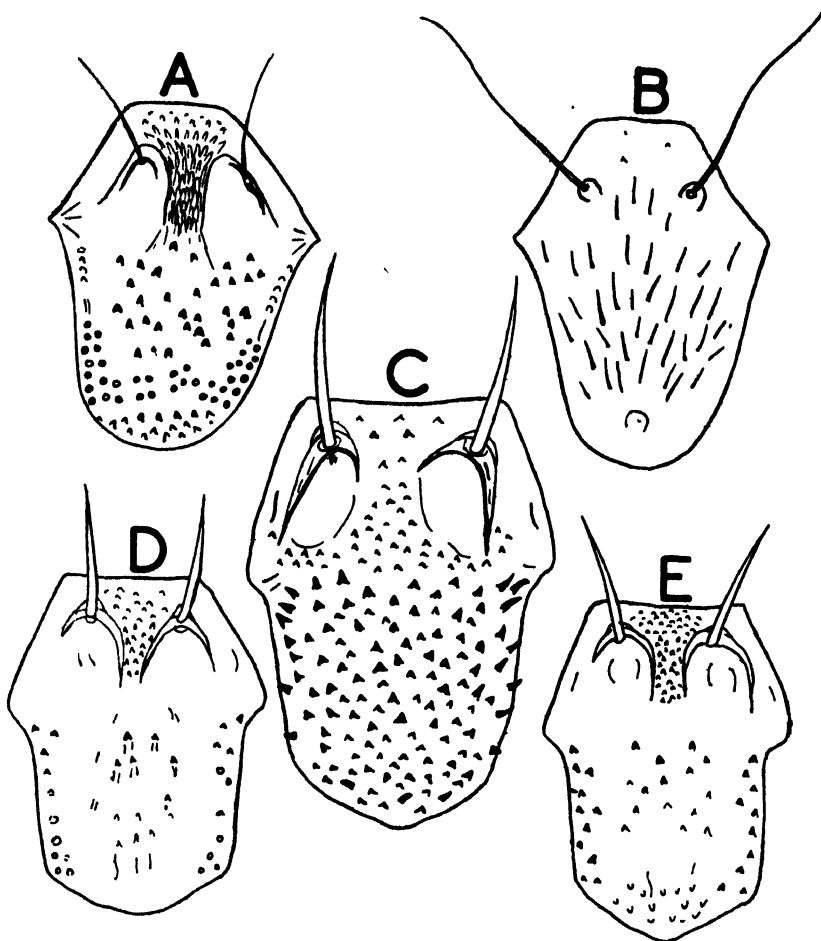


FIG. 9. Opercula of male pupae of *Culicoides* spp. ( $\times 225$ ). A.—*C. impunctatus*; B.—*C. obsoletus*; C.—*C. odibilis*; D.—*C. pallidicornis*; E.—*C. cubitalis*.

is prominent and bears two long fine bristles, and the ventrolateral tubercle is small and also bears two long fine bristles. The ventromedian tubercle is inconspicuous and bears two delicate setae.

There are four dorsal tubercles (fig. 11), the most anterior of which (1) is large and bears a spine; the tubercle posterior to this (2) is double and bears a longer finer spine than the more anterior tubercle. Dorsal tubercles 3 and 4 are smaller, the third bearing a long fine bristle and the fourth a short spine. The posterodorsal tubercle is small and bears a single long hair.

The arrangement of the tubercles on the abdominal segments three to seven is as follows :

	Dorsal					Lateral			Ventral		
Anterior submarginal (a.s.m.)		×		×				^			
Posterior marginal (p.m.) ...	×	×	×	×	×	×	×	×	×	×	×
	5	4	3	2	1	3	2	1	3	2	1

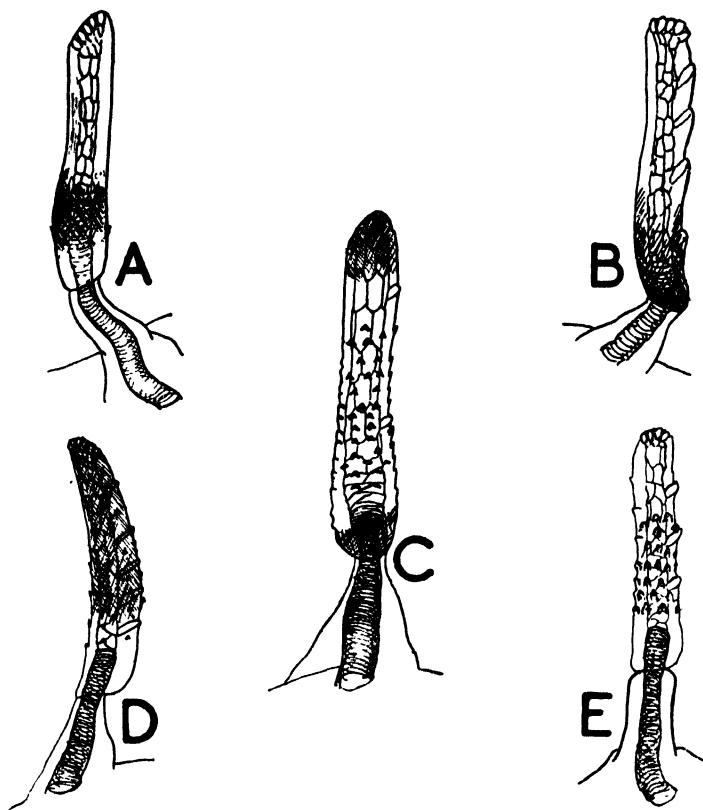


FIG. 10. Respiratory trumpets of male pupae of *Culicoides* spp. ( $\times 225$ ). A.—*C. impunctatus*; B.—*C. obsoletus*; C.—*C. odibilis*; D.—*C. pallidicornis*; E.—*C. cubitalis*.

The tubercles, which have a single point, appear to vary a little with individual specimens, but typically their structure is as follows : l.a.s.m. tubercle 1 bears a small spine ; d.a.s.m. tubercle 2 bears a long bristle ; d.a.s.m. tubercle 4 bears a small spine ; v.p.m. tubercle 1 bears a very small spine ; v.p.m. tubercle 2 bears a long bristle ; v.p.m. tubercle 3 bears a small spine ; l.p.m. tubercle 1 bears a small spine ; l.p.m. tubercle 2 bears a long bristle ; l.p.m. tubercle 3 bears a small spine ; d.p.m. tubercle 1 bears a

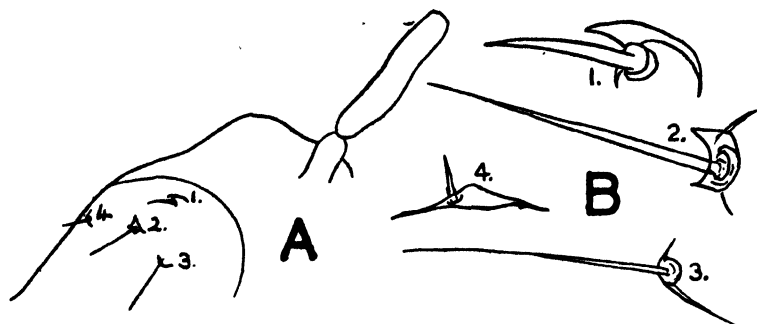


FIG. 11. A.—Showing the position of the dorsal tubercle on the thorax of the pupa of *C. impunctatus*; B.—Dorsal tubercles 1, 2, 3 and 4 ( $\times 675$ ).

long bristle; d.p.m. tubercle 2 bears a small spine; d.p.m. tubercles 3 and 4 bear neither spine nor bristles; and d.p.m. tubercle 5 bears a very small spine (fig. 12).

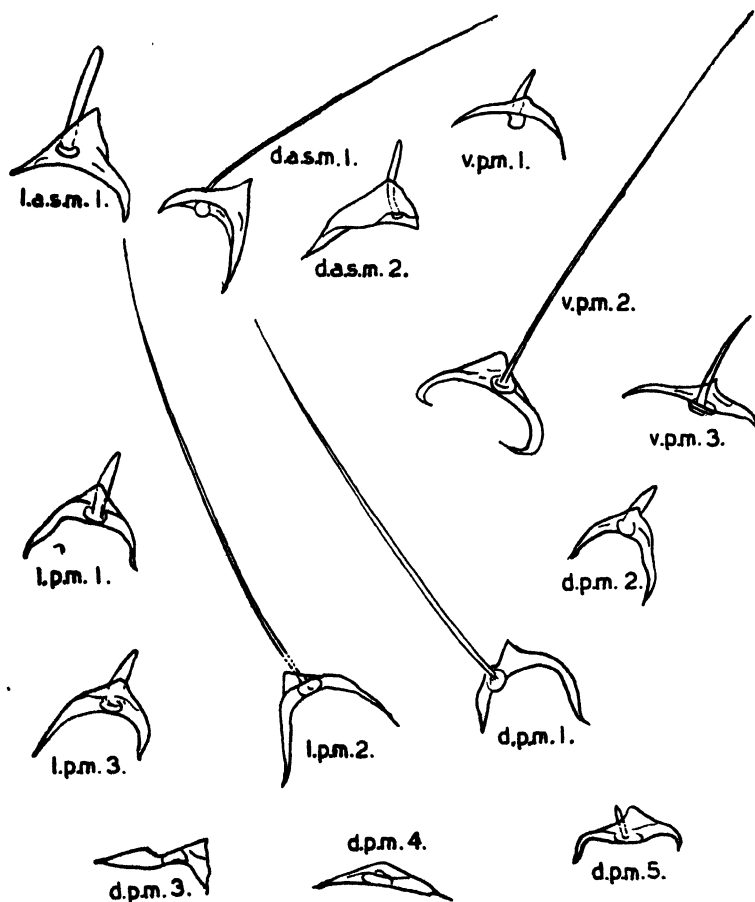


FIG. 12. The tubercles on the fourth abdominal segment of the pupa of *C. impunctatus* ( $\times 675$ ).

(2) *C. obsoletus*(a) *The Incidence of Pupae in One Site Throughout a Complete Year*

Sampling of plot 'B' for larvae and pupae was carried out between April, 1945, and April, 1946, in a similar manner to that in which plot 'A' was sampled. The results of sampling for larvae will be described later.

The first pupae were recovered from plot 'B' during the period ending on April 28th, and the average numbers of all species of pupae recovered per sample reached a peak during the two-weekly period ending on July 21st. This is shown in Table IV and graph 4. The last pupae were recovered during the period ending on September 28th, which corresponded with the finding of the last fully grown larvae.

Unlike plot 'A,' which proved to be a 'pure' breeding-ground of *C. impunctatus*, plot 'B' was found to be the breeding-site of four species, namely, *C. obsoletus*, *C. odibilis*, *C. pallidicornis* and *C. cubitalis*. All these species, except *C. pallidicornis* and *C. cubitalis*, had slightly different emergence periods, as is shown in graph 5, but each will be discussed separately later.

TABLE IV

The total numbers of pupae of various species of *Culicoides* recovered from six samples taken from plot 'B' every two-weekly period between April, 1945, and April, 1946

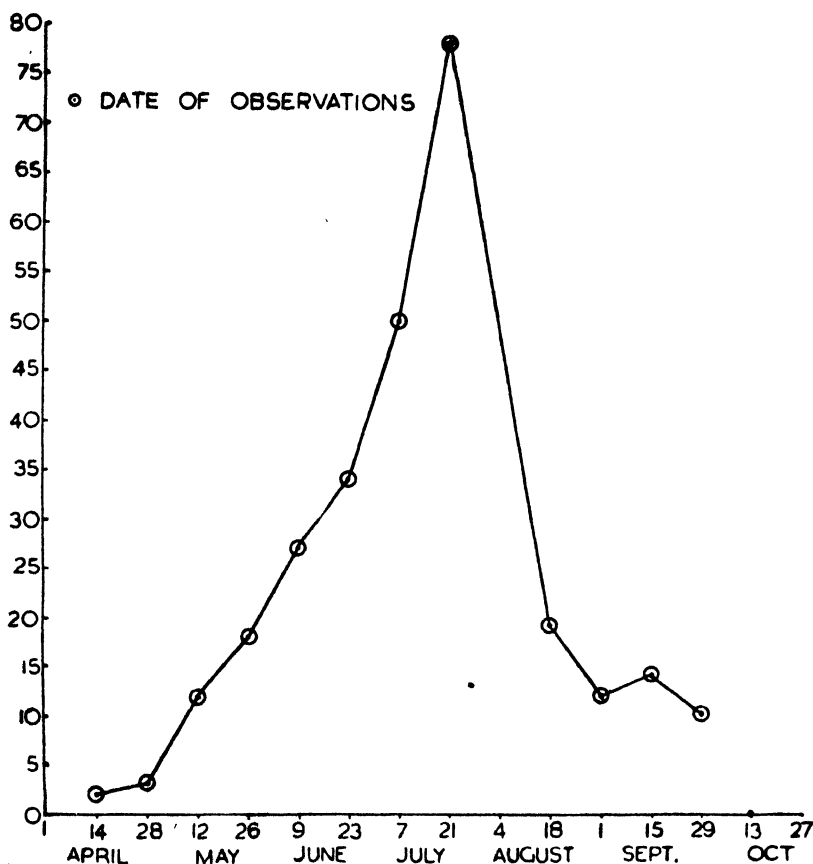
Two-weekly periods ending	Total no. of pupae from six samples	<i>C. obsoletus</i>	<i>C. pallidicornis</i>	<i>C. cubitalis</i>	<i>C. odibilis</i>
14.4.45	2	2	0	0	0
28.4.45	3	3	0	0	0
12.5.45	12	12	0	0	0
26.5.45	18	18	0	0	0
9.6.45	27	15	3	9	0
23.6.45	34	6	8	13	7
7.7.45	50	3	4	18	25
21.7.45	78	2	6	19	51
4.8.45	—	—	—	—	—
18.8.45	19	2	1	2	14
1.9.45	12	12	0	0	0
15.9.45	14	14	0	0	0
29.9.45	10	10	0	0	0
13.10.45	0	0	0	0	0

The first pupae recovered during the period ending on April 14th were those of *C. obsoletus*, and pupae of this species formed the bulk of the pupae recovered up to the period ending on May 9th. After the period ending on June 23rd very few pupae of *C. obsoletus* were recovered until the period ending on September 1st, and from that time onwards, until September 29th, there was a second peak in the abundance of the pupae of *C. obsoletus*. This double emergence period confirms the evidence obtained by laboratory breeding, by breeding under field conditions, and by catches of wild flies—the results of which are described below—that the strain of *C. obsoletus* breeding in Knowsley Park normally has two generations per year.

(b) *The Incidence of Adults in One Site Throughout a Complete Year*

Throughout the summer of 1945, catches of female *C. obsoletus* were made as they alighted with females of *C. impunctatus* on a black cloth. The numbers obtained on various dates are recorded in graph 2 and Table II.

The first specimens of *Culicoides* were caught as early as April 3rd and were identified as *C. obsoletus*. Small numbers of this species were caught throughout the rest of April, but at the beginning of May the numbers of the species alighting on the cloth began to increase markedly. They increased throughout May and reached a peak during the first half of June. Throughout the second half of June the numbers began to decrease, and comparatively small numbers of this species were caught throughout July and August. The numbers of *C. obsoletus* alighting on the cloth began to increase again at the beginning of September, and a second peak of abundance occurred at the end of that month. During October the number of *C. obsoletus* again began to decline, the last specimens being caught



GRAPH 4. The total numbers of *Culicoides* pupae recovered from six samples taken from plot 'B' every two-week period between April, 1945, and April, 1946.

on the 23rd. This second peak of abundance, which confirms Edwards' (1939) statement that *C. obsoletus* may be abundant again in the autumn, suggests that *C. obsoletus* has two generations per year, a fact which is confirmed by laboratory breeding and breeding under field conditions.

Confirmatory catches made during the summer of 1946 agree with the records made during 1945, with one discrepancy only—the peak of abundance at the beginning of June was slightly higher in 1946 than in 1945.

### (c) *The Life-Cycle as Studied in the Laboratory*

The technique which was used for feeding *Culicoides* in the laboratory has been described. *C. obsoletus* caught in one evening were given an opportunity to feed the following day, and as many as 96 per cent. have been recorded as taking a blood-meal then. It appeared to the author that this species fed slightly more readily on the rabbit's ear than did *C. impunctatus*. Subsequent attempts to induce the flies to take a blood-meal resulted in smaller percentages feeding with each successive opportunity given. No flies would take a blood-meal on the day prior to oviposition. Individuals of this species have been recorded as taking five blood-meals. However, the number of blood-meals taken does not appear to influence the number of eggs laid, similar numbers being laid after one blood-meal as after taking more than one. As with *C. impunctatus*, the time taken for individuals to become fully gorged varies; in the case of *C. obsoletus* individuals have taken up to 40 minutes before being fully replete, and a small proportion seem to have difficulty in taking a blood-meal, since they remain with proboscis inserted for 45 minutes, at the end of which time no trace of blood is found in the gut.

Wild caught flies laid eggs between one and 18 days after capture, indicating that the normal period taken for development of the eggs is about two and a half weeks and that eggs laid at shorter intervals were already developing when the parent female was caught and given its first blood-meal in the laboratory. Females of *C. obsoletus* have been recorded as laying between 35 and 120 eggs in one batch, the average number deposited being in the region of 75. On no occasion has the author obtained a second batch of eggs from a fly of this species. After oviposition, which as a rule was complete, the females always died. The eggs were usually laid in small groups or in a double row on damp filter-paper in the laboratory; they have not been observed in their natural habitat. Hatching occurred in the laboratory from 30 hours to 11 days after oviposition, but the average time taken was three days. The method of hatching differs from that of the eggs of *C. impunctatus* and from that of the eggs of *C. odibilis*, the only other species of *Culicoides* eggs observed by the writer. A very small anterior cap splits off, and simultaneously a longitudinal split occurs anteroposteriorly; this split may extend along almost the whole length of the dorsal side of the egg, although the exact length of the split varies considerably even in the same batch of eggs. The first stage larvae take up to two minutes to free themselves from the egg-shell and their movement is extremely sluggish.

The length of the larval stage of *C. obsoletus* in the laboratory varies and may last up to five months, but the majority of the larvae pupated within three and a half months. The larvae pupated in the drier more superficial layers of the breeding-medium, and the pupal stage was usually of five days' duration. The first flies to hatch were males, but the number of adults emerging, in relation to the number of eggs transferred to the breeding-pots, was only 15 per cent. The above account concerns larvae kept in the laboratory, not a single fly emerging from the breeding-pots kept in the insectarium at 23-24° C.

Flies hatched in the laboratory would copulate in the confinement of a lamp-glass, although, as in the case of *C. impunctatus*, this was never observed, the statement being based on the finding of spermatozoa in the spermathecae of the females. Fertilization would appear to be independent of a blood-meal, which is usually taken two or three days after emergence. On one occasion only did a female of this species reared in the



laboratory survive long enough for complete development of the ovaries; this female laid 72 eggs 17 days after taking its first blood-meal and 19 days after emerging.

(d) *The Life-Cycle as Studied in the Field*

*C. obsoletus* was maintained under field conditions by means of a technique similar to that used for rearing *C. impunctatus*. From one batch of 74 eggs laid on May 15th, 1945, one male hatched on August 12th, 1945, and 10 males and nine females between September 29th and October 10th, 1945. From six other batches of eggs laid in June, adults hatched at the beginning of October. However, from eggs laid in August, 1945, no adults were recovered until May, 1946. These observations would indicate that *C. obsoletus* normally has two broods each season.

(e) *Its Habits*

(i) *Hours of Activity*. The results of catches of *C. obsoletus* made on a black cloth on different occasions from 11 a.m. G.M.T. until sunset are recorded in graph 3, A.

*C. obsoletus* also are most active during the evening. Like *C. impunctatus* they are not very much in evidence during the day, but they become more numerous about three and a half hours before sunset. Their period of greatest activity begins slightly earlier than that of *C. impunctatus*, and the first midges of a catch extending over several hours before sunset are invariably *C. obsoletus*. Up to about two hours after sunset they tend to be far more numerous than *C. impunctatus*, though after that time the numbers of the latter species are far in advance of those of *C. obsoletus*. Towards sunset the numbers of *C. obsoletus* tend to decrease, and we have no records of the activity of the species during the hours of darkness.

(ii) *Flight Range*. Records regarding the flight range of *C. obsoletus* have not been made, principally because of the inadequacy of our knowledge concerning the breeding-grounds of the species. However, we have no evidence which suggests that *C. obsoletus* normally occurs at great distances from its breeding-ground.

(iii) *Range of Breeding-Grounds*. Larvae and pupae of *C. obsoletus* have been recovered from a variety of different situations, all with an acid reaction. They have been recovered from plot 'B,' which has been described, from decaying leaves in a tree-hole and under a hedge bank, and from a shady ditch full of rotting leaves. However, from such records as we have concerning the breeding-grounds of *C. obsoletus* we have been unable to form any definite opinion as to what are the essential features of the breeding-grounds of the species, since on several occasions from what would appear an extremely likely breeding-ground we have recovered no immature stages. However, the peaty soil characteristic of the breeding-grounds of *C. impunctatus* would not appear to be characteristic of the breeding-grounds of *C. obsoletus*.

(f) *The Morphology of the Adult, Together with an Account of the Egg, Larva and Pupa*

References to descriptions of adult females of *C. obsoletus* have already been given, and Edwards (1939) and Root and Hoffman (1937) have described and drawn the male genitalia. We have nothing to add to these descriptions.

The eggs of *C. obsoletus* (fig. 4, D) have an average length of about  $380\mu$  and a breadth

of  $76\mu$  one hour after being laid, but the length varies between  $310\mu$  and  $410\mu$ . They are cigar-shaped, and the posterior end is more markedly pointed than the anterior. When first laid they are light in colour, but they assume a dark greyish-black colour within half an hour. The chorion has sucker-like structures over its whole surface, arranged in ill-defined single rows. These sucker-like structures (fig. 4, E) are borne on longer stalks than those of the eggs of *C. impunctatus*, a few eggs being found in which these structures were as long as half the width of the egg. The first stage larva has a length of about 0.5 mm. and a creamy-white head measuring  $77\mu$  long and  $70\mu$  wide.

The larvae of *C. obsoletus*, when mature, have an average length of about 4.5 mm. and are semi-transparent, with a yellowish head which is much less heavily chitinized than that of the fully grown larvae of *C. impunctatus*. The average length of the head is  $217\mu$  and its breadth  $164\mu$ , being broader in proportion to its length than is the head capsule of the larva of *C. impunctatus*.

The arrangement of the bristles on the capsule appears to be as in *C. impunctatus*. The structure of the antennae, labrum and maxillae is extremely difficult to observe, and although differences do occur in different species such differences as there are would appear to be of little practical use for identification purposes. The mandibles are heavily chitinized, pointed and curved, and bear a long finger-like tooth at about the distal third (fig. 8, B).

The labial plate is fairly heavily chitinized and bears three or four minute teeth on either side. The hypopharyngeal sclerite, however, appears to afford the most practical method of identifying the larva (fig. 8, B). Dorsally this bears six large blunt teeth on either side, and ventral to these are three rows of fine pointed teeth. Two rows of teeth, the dorsal one coarser than the ventral, extend along the lateral wings. The larva of this species has two pairs of deeply cleft retractile gills surrounding the anus.

The pupa of *C. obsoletus* is about 2.0 mm. in length. The operculum (fig. 9, B) is covered with long hair-like bristles, and the respiratory trumpets (fig. 10, B) are short, straight and raised on a stalk, which is about one-fifth of the length of the trumpet. The length of the trumpet is about  $165\mu$  and that of the stalk about  $34\mu$ . The trumpet is darker at its base and bears four spiracular openings raised on blunt protuberances arranged at an approximately equal distance along its length on its median surface. Distally there is a fan-like group of 4-6 more spiracular openings. It bears neither scales nor spines.

The anteromarginal tubercle is small and rounded and bears a bristle almost as long as the respiratory trumpet. The anterodorsal tubercle is large and blunt and bears two long bristles, the outer one also almost as long as the respiratory trumpet. The dorso-lateral tubercle is fairly prominent and bears two long fine bristles, and the ventrolateral tubercle is small and also bears two long fine bristles. The ventromedian tubercle is inconspicuous and bears two delicate setae.

There are four dorsal tubercles, the most anterior of which is large and bears a spine; the tubercle posterior to this tends to have a double base. The other two are smaller, one bearing a fine bristle and the more dorsal one a short spine. The postero-dorsal tubercle is small and bears a hair.

The arrangement of the tubercles on the abdominal segments three to seven is as follows :

	Dorsal					Lateral			Ventral		
Anterior submarginal (a.s.m.)		×	×	×				×			
Posterior marginal (p.m.) ...				×	×	×	×	×	×	×	×
	5	4	3	2	1	3	2	1	3	2	1

The spines and bristles borne by the tubercles are arranged as in *C. impunctatus*, but there are three dorsal anterior submarginal tubercles instead of two, and there are only two dorsal posterior marginal tubercles as opposed to five in *C. impunctatus*.

### (3) *C. odibilis*, *C. pallidicornis* AND *C. cubitalis*

#### (a) *The Incidence of Pupae in One Site Throughout a Complete Year*

As already stated, *C. odibilis*, *C. pallidicornis* and *C. cubitalis* hatched from pupae recovered from plot 'B.' The actual numbers of pupae of these species recovered from six samples are recorded in Table IV and graph 5.

*C. odibilis* has the latest and the most defined emergence period of all the species recovered from the site. The first pupae of this species were recovered during the period ending on June 23rd, and the last were recovered during the period ending on August 18th. They reached their peak of abundance during the middle of July.

It will be seen that *C. pallidicornis* and *C. cubitalis* had similar emergence periods. They reached their peak of abundance during the end of June and the first three weeks in July, and no pupae of these species were recovered after the period ending on August 18th.

All three species have limited emergence periods. This, in addition to the fact that larvae of the three species have been kept in the laboratory for periods of up to four months before they pupated, indicates that all have one cycle per year.

#### (b) *The Morphology of the Adult and Immature Forms*

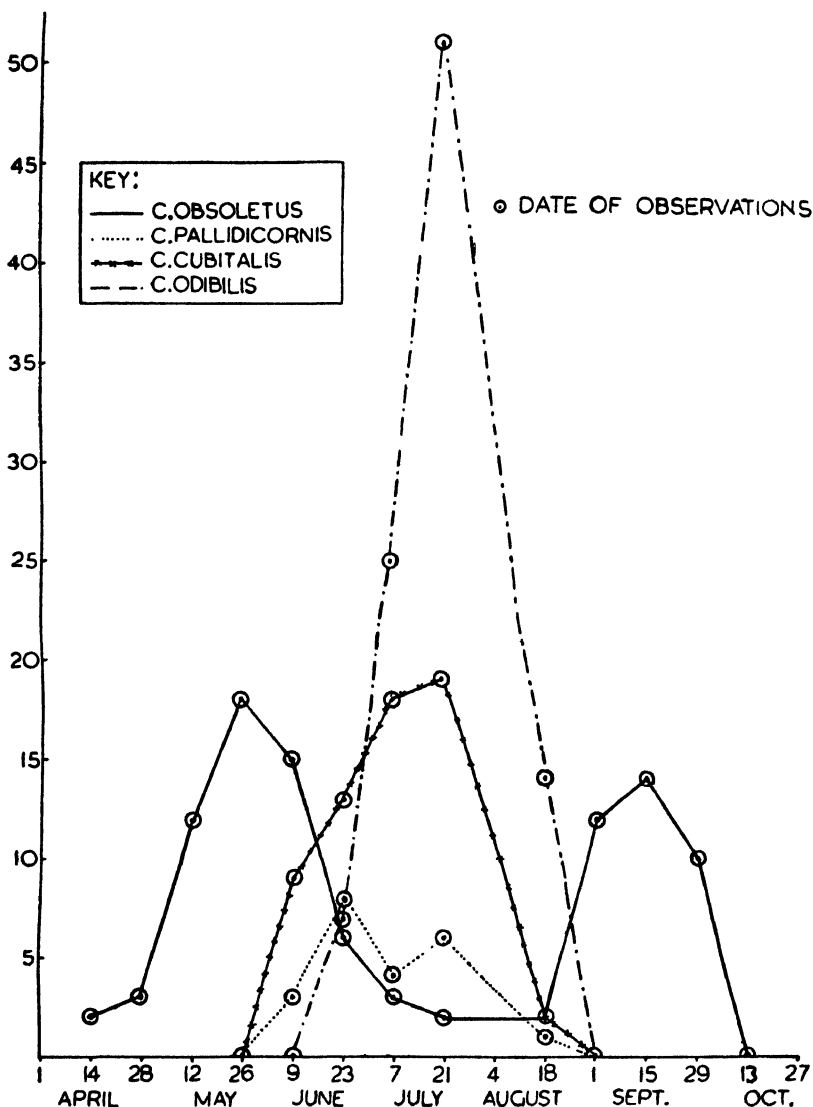
##### (i) *C. odibilis*

The adult of this species has been adequately described, and references have already been given to these descriptions; however, we think it necessary to give a more detailed drawing of the male genitalia (fig. 3, B) than that of Edwards (1939).

**Egg** (fig. 4, F). Females of *C. odibilis* lay an average of 80 eggs in one batch. These are laid singly or in small masses of up to 30 eggs lying side by side but not all orientated in the same direction. Their average length is  $370\mu$  and their breadth  $65\mu$  when they are just laid. They are pale cream in colour and are covered with rows of small sucker-like structures, each row having smaller sucker-like structures on either side of it, giving the appearance of three rows. Both anterior and posterior ends are rounded but the anterior end more so than the posterior. Within an hour of being laid the eggs swell to an average size of  $400\mu$  in length and  $70\mu$  in breadth. They also darken in colour, becoming brownish, and the posterior end becomes more markedly pointed, the egg becoming rather more banana- than sausage-shaped.

On an average, eggs of *C. odibilis* hatch within three days of being laid, and the eye-spots of the larva may be seen through the chorion prior to hatching. The method of splitting of the chorion differs from that of both *C. obsoletus* and *C. impunctatus*. A

small cap splits off at the anterior end of the egg, and simultaneously a longitudinal split appears along the mid-dorsal line of the chorion. This split extends posteriorly for about one-third of the length of the egg and curves slightly to the right or left. The cap may or may not—usually it does not—separate with the head of the emerging larva, which takes approximately two minutes to free itself. The first stage larvae have a length of



GRAPH 5. The total numbers of pupae of various species of *Culicoides* recovered from six samples taken from plot 'B' every two-weekly period between April, 1945, and April, 1946.

0.66 mm. and a cream-coloured opaque head which is  $70\mu$  long and  $61\mu$  broad. They are almost transparent, but can be seen with the naked eye swimming actively, their

movement being very swift when compared with the sluggish movement of the first stage larvae of *C. obsoletus*.

The larvae of *C. odibilis*, when mature, have an average length of about 6.0 mm. They are semi-transparent, and have a pale yellow lightly chitinized head, the average measurements of which are  $190\mu$  by  $132\mu$ .

The head differs from that of the mature larva of *C. impunctatus* in several details, but the available larval material of this species, as of the larvae of *C. pallidicornis* and *C. cubitalis*, consisted of pelts only, which is unsatisfactory for descriptive purposes, complete larvae being necessary for the basis of an adequate description.

The arrangement of the bristles on the head capsule appears to be as in *C. impunctatus*. The antennae have two large lobes, each bearing a spine, and four small lobes. The mandibles (fig. 8, C) are not very heavily chitinized, and bear a finger-like blunt tooth which rises about half-way along their length. The hypopharyngeal sclerite (fig. 8, C) is also not heavily chitinized and bears a double row of about seven pointed teeth posteriorly on either side. The labial plate is lightly chitinized and does not appear to be notched. The terminal segment of the larva has two pairs of deeply cleft retractible gills surrounding the anus.

The pupa of *C. odibilis* is about 2.75 mm. in length. The operculum (fig. 9, C) is densely covered posteriorly with sharp short spines. Towards the edges these spines are larger and longer. Anteriorly the spines merge into blunt protuberances. The respiratory trumpets (fig. 10, C) are slightly curved and approximately  $216\mu$  in length, raised on a stalk about  $70\mu$  in length. The main tracheal trunk is stouter than that of other species of pupae examined, and bears three or four lateral branches, two (plus a possible third) occurring along the proximal third of the trumpet, and the other about a third of the length of the trumpet from its distal end. The tracheal trunk ends in five or six short branches. The proximal two-thirds of the trumpet almost to its base is densely covered with sharp spines, and the immediately proximal and distal ends are darkened.

The anteromarginal tubercle is very prominent and bears a very stout spine. The anterodorsal tubercle is also prominent, conical, and bears two spines, the inner one small and the outer one longer and stouter; the stouter of these two spines is just over half as long as the anteromarginal spine. The dorsolateral tubercle is prominent and bears two long bristles and a short stout spine; the ventrolateral tubercle is represented only by one fairly long and another shorter hair. The posterodorsal tubercle is small and bears a fine hair.

There are four dorsal tubercles. The most anterior of these is large and double and bears a stout spine. The tubercle just posterior to this is also double, but smaller, and it also bears a stout, but smaller, spine. The third and fourth tubercles are small, the third bearing a long fine bristle and the fourth a short fine seta.

The formula for the tubercles on the abdominal segments three to seven is similar to that of *C. impunctatus*. As in the other species of pupae examined, the tubercles appear to vary a little in individual specimens. They are broad-based, and typically their structure is as follows: l.a.s.m. tubercle 1 bifid, bears a spine; d.a.s.m. tubercle 2 bifid, bears a long bristle; d.a.s.m. tubercle 4 bifid, bears a spine; v.p.m. tubercle 1 small, bears a short spine; v.p.m. tubercle 2 small, bears a larger finer spine; v.p.m. tubercle 3 small, bears a short spine; l.p.m. tubercle 1 large and bifid, bears a stout spine; l.p.m.

tubercle 2 very large and bifid, bears a long bristle ; l.p.m. tubercle 3 large and bifid, bears a stout spine ; d.p.m. tubercle 1 bifid, bears a long bristle ; d.p.m. tubercle 2 smaller, bears a spine ; d.p.m. tubercle 3 small, bears no spine or bristle ; d.p.m. tubercle 4 small, bears no spine or bristle ; d.p.m. tubercle 5 small, bears a rudimentary spine.

(ii) *C. pallidicornis*

References to descriptions of the *adults* of this species have already been cited ; however, we have given a more detailed drawing of the male genitalia (fig. 3, C) than that of Edwards (1939).

The *larvae* of *C. pallidicornis*, when mature, have an average length of 5.5 mm. They are semi-transparent, and have pale yellow heads which are not heavily chitinized. The average length of the head of larvae of this species is  $200\mu$  and the breadth  $140\mu$ .

Structurally the larvae of this species differ from those of *C. impunctatus* in the following details. The more posterior of the two pairs of bristles situated posteriorly on the epicranial plates is rudimentary, and the pair anterior to these two pairs of posterior epicranial bristles is also rudimentary. The mandibles (fig. 8, D) are relatively shorter and blunter than those of *C. impunctatus*, and the tooth situated near the distal third is also blunter. The hypopharyngeal sclerite (fig. 8, D) is quite heavily chitinized and bears dorsally a row of five or six sharp triangular teeth on each side of the posterior margin. With the limited material at our disposal, however, we have been unable to discern whether or not there is a second row of teeth ventrally. The labial plate is moderately heavily chitinized and is slightly more pointed anteriorly than that of *C. impunctatus*. At its tip is a row of about 20 extremely fine teeth.

The *pupa* of *C. pallidicornis* is about 1.82 mm. in length. The operculum (fig. 9, D) is sparsely covered centrally with blunt spines, which towards each edge form a more definite row, merging into blunt processes ventrally. Anteriorly between the antero-marginal tubercles the operculum is densely covered with fine blunt processes. The respiratory trumpets (fig. 10, D) are fairly long, slightly curved and raised on a stalk. Their average length is  $170\mu$  and that of the stalk  $50\mu$ . The main tracheal trunk bears from four to six lateral branches, ending in a similar number of knob-like processes. Their distribution along the length of the trumpet, however, appears to vary individually. The tracheal trunk ends in a fan-like group of four, five or six branches. The distal three-quarters of the trumpet is darkish, and the proximal two-thirds is sparsely covered with spines.

The anteromarginal tubercle is prominent and bears a stout spine. The anterodorsal tubercle is prominent and bears two spines, one smaller than the other. The dorsolateral tubercle bears a hair and a spine, and the ventrolateral tubercle is very small and bears two long fine bristles. The ventromedian tubercle is inconspicuous and bears two fine short bristles. The posterodorsal tubercle is small and bears a single long hair.

There are four dorsal tubercles, the most anterior of which is the largest ; it is bifid and bears a stoutish spine. The second is smaller, also bifid, and bears a stoutish spine. The third and fourth are smaller, the third bearing a long fine bristle and the fourth a short spine.

The formula for the tubercles, which are broad-based and tend to be bifid, on abdominal segments three to seven is similar to that of *C. impunctatus*. Typically the structure of the tubercles is as follows : l.a.s.m. tubercle 1 bifid, bears a spine ; d.a.s.m.

tubercle 2 bifid, bears a long bristle; d.a.s.m. tubercle 4 bifid, bears a spine; v.p.m. tubercle 1 bears a spine; v.p.m. tubercle 2 bifid, bears a long bristle; v.p.m. tubercle 3 bifid, bears a spine; l.p.m. tubercle 1 large and bifid, bears a spine; l.p.m. tubercle 2 large and bifid, bears a long bristle; l.p.m. tubercle 3 large and bifid, bears a long spine; d.p.m. tubercle 1 bifid, bears a long bristle; d.p.m. tubercle 2 bifid, bears a spine; d.p.m. tubercle 3 small, bears no spine or bristle; d.p.m. tubercle 4 small, bears no spine or bristle; d.p.m. tubercle 5 small, bears a very small spine.

(iii) *C. cubitalis*

The male of this species has been described by Edwards (1939), but we have given a more detailed drawing of the male genitalia (fig. 3, D). The female has not previously been identified with certainty.

The female of this species is similar to the female of *C. pallidicornis*. The wing has two white patches on the front margin, separated by a blackish spot on the radial cells, as in *C. fascipennis* Staeg. and *C. pallidicornis*, but, as noted by Edwards (1939) in the male of the species, the two spots are about equal in size and are both fairly broad. Two further pale areas, both indistinct, are visible on each side of the lower branch of the cubital fork when the wing is viewed obliquely against a dark background. Both these pale areas are absent in the female *C. pallidicornis*. In addition, there is a very faint pale streak between the media and the cubitus, but no trace of pale spots round the apex of the wing. The basal cell is bare of hairs, as in *C. pallidicornis*, but the rest of the wing is definitely more hairy than the wing of that species. The average wing-length is about 1.45 mm. The thorax is greyish-brown and devoid of markings, and the scutellum is yellowish. The legs are pale grey, with the joint between the femur and tibia blackish.

The larvae of *C. cubitalis*, when mature, have an average length of 6.0 mm. They are semi-transparent, and have pale yellow lightly chitinized heads. The average length of the head of larvae of this species is 180  $\mu$  and the breadth 140  $\mu$ .

Structurally the larvae of this species differ from those of *C. impunctatus* in the following details. The more posterior of the two pairs of bristles situated posteriorly on the epicranial plate is small; the tooth on each of the mandibles (fig. 8, E) is longer and resembles that of *C. odibilis*; the hypopharyngeal sclerite (fig. 8, E) is moderately heavily chitinized and bears dorsally on the posterior margin two plates, each bearing a row of eight or nine blunt teeth. Ventral to these there is a second row of teeth, and there is also a row of small teeth in the lateral wings. The labial plate is moderately heavily chitinized but does not appear to bear teeth.

The pupa of *C. cubitalis* is about 2.0 mm. in length. The operculum (fig. 9, E) is sparsely covered centrally with spines, which towards each edge of the operculum form a more definite row. These spines are slightly denser than those on the operculum of *C. pallidicornis*, but, as with other species, their density varies a little individually. Anteriorly between the anteromarginal tubercles the operculum is densely covered with fine blunt processes. A group of blunt processes is situated ventrally. The respiratory trumpets (fig. 10, E) are slightly curved and are shorter than those of *C. pallidicornis*, their average length being 150  $\mu$ , with a relatively slightly longer stalk of 50  $\mu$ . The main tracheal trunk has three or four lateral branches evenly spaced along its length, and ends in four or five branches arranged fan-wise. The trumpet is light in colour along its

whole length, and its proximal two-thirds, except for a small area near its base, is densely covered with large scales.

The anteromarginal tubercle is prominent and bears a stout spine. The anterodorsal tubercle is prominent and bears two spines, one smaller than the other. The dorsolateral tubercle bears a hair and a spine, and the ventrolateral tubercle is very small and bears two long fine bristles. The ventromedian tubercle is very inconspicuous and bears one long and one short fine bristle. The posterodorsal tubercle is small and bears a long hair.

There are four dorsal tubercles. The most anterior is slightly bifid and bears a stout spine. The tubercle behind this (2) is slightly larger, tending to be bifid, and also bears a stout spine. Tubercles 3 and 4 are small, the third bearing a long fine bristle and the fourth a short spine.

The formula for the tubercles on the abdominal segments three to seven is similar to that of *C. impunctatus*. Typically the structure of the tubercles, which are broad-based but with little or no tendency towards bifurcation, is as follows: l.a.s.m. tubercle 1 bears a long bristle; d.a.s.m. tubercle 1 bears a long bristle; d.a.s.m. tubercle 2 bears a fine spine; v.p.m. tubercle 1 bears a very small spine; v.p.m. tubercle 2 bears a long bristle; v.p.m. tubercle 3 bears a small fine spine; l.p.m. tubercle 1 bears a spine; l.p.m. tubercle 2 large, bears a long bristle; l.p.m. tubercle 3 large, bears a spine; d.p.m. tubercle 1 bears a long bristle; d.p.m. tubercle 2 bears a spine; d.p.m. tubercle 3 bears no spine or bristle; d.p.m. tubercle 4 bears no spine or bristle; d.p.m. tubercle 5 bears a rudimentary spine.

#### (4) *C. chiopterus*

##### *The Incidence of Adults in One Site Throughout a Complete Year*

During the collection of female *C. impunctatus* and *C. obsoletus* on the black cloth small numbers of *C. chiopterus* were caught. These are recorded in graph 2.

The first specimens of this species were caught on May 7th and the last on September 19th; they reached a peak of abundance in June.

#### (5) THE INCIDENCE OF UNKNOWN LARVAE IN ONE SITE THROUGHOUT A COMPLETE YEAR

As recorded above, sampling of plot 'B' for larvae and pupae was carried out between April, 1945, and April, 1946. From the pupae recovered four different species of *Culicoides* hatched, namely, *C. obsoletus*, *C. odibilis*, *C. pallidicornis* and *C. cubitalis*. Without microscopical examination, however, it is impossible to separate the larvae of these species, and the larvae recovered were therefore recorded as a whole and divided arbitrarily into small, medium and large. As with the larvae of *C. impunctatus*, we believe that these three groups represent second, third and fourth instar larvae of the species concerned. The results, which are recorded in Table V, histogram 2 and graph 6, are not essentially different from those obtained from the sampling of plot 'A.'

As in plot 'A,' from what must be regarded as a steady level the average numbers of larvae recovered per sample from plot 'B' began to fall at the beginning of May. This fall—which is, of course, accounted for by the appearance of pupae at that time



—continued until the two-weekly period ending on July 7th, and from the two-weekly period ending on July 21st until the period ending on September 15th there was a very marked rise in the abundance of larvae, the average number recovered per sample increasing from 29.33 to 111.16, the maximum recorded throughout the 12 months' observations. The numbers of larvae recovered between September 16th and October 13th showed a decrease on the numbers obtained during the period September 2nd to 15th, but during the following six weeks, i.e., from October 14th to November 24th, a second

TABLE

The average numbers of larvae, of all stages, and the proportions of small, half-grown and fully grown larvae 1945, and

Two-weekly period ending	No. of samples examined	Total no. of larvae recovered from six samples	Average no. of larvae recovered from each sample	Total no. of small larvae recovered from six samples	Percentage of small larvae in total recovered from six samples	Average no. of small larvae recovered from each sample	Total no. of half-grown larvae recovered from six samples
14.4.45	6	309	51.5	0	0	0	0
28.4.45	6	331	55.16	0	0	0	0
12.5.45	6	264	44.0	0	0	0	0
26.5.45	6	285	47.5	3	1.05	0.5	0
9.6.45	6	218	36.3	17	7.8	2.8	0
23.6.45	6	192	32.0	27	14.0	4.5	0
7.7.45	6	176	29.33	45	25.6	7.5	3
21.7.45	6	210	35.0	84	40.0	14.0	30
4.8.45	—	—	—	—	—	—	—
18.8.45	6	378	63.0	293	77.5	48.8	51
1.9.45	6	622	103.66	509	81.83	84.8	86
15.9.45	6	607	111.16	491	73.6	81.8	152
29.9.45	6	550	91.66	316	57.4	52.66	227
13.10.45	6	488	81.3	177	36.3	29.5	311
27.10.45	6	513	85.5	90	17.5	15.0	423
10.11.45	6	529	88.16	73	13.8	12.16	473
24.11.45	6	575	95.8	87	15.1	14.5	421
8.12.45	6	508	84.3	37	7.3	6.16	283
22.12.45	6	451	75.16	0	0	0	155
5.1.46	6	482	80.3	0	0	0	66
19.1.46	6	425	70.8	0	0	0	55
2.2.46	6	394	65.66	0	0	0	9
16.2.46	6	356	59.3	0	0	0	0
2.3.46	6	384	64.0	0	0	0	0
16.3.46	6	333	55.5	0	0	0	0
30.3.46	6	360	60.0	0	0	0	0
13.4.46	6	299	49.8	0	0	0	0

increase occurred in the numbers of larvae recovered per sample. In the fact that *C. obsoletus* has two generations per year lies a possible explanation of the increase in the incidence of larvae throughout October and November in plot 'B.' This was possibly due to an influx of young larvae hatched from eggs laid by females of *C. obsoletus* emerging in September. Certainly during this period the numbers of small larvae recovered kept at a steady level, as opposed to the drop in their numbers which could be expected at this time. Between the periods ending on December 8th, 1945, and April 13th, 1946, there was a steady decrease in the average number of larvae recovered per sample, the level reached in April, 1946, approximating to that in April, 1945.

## VI. SUMMARY

1. The literature is reviewed concerning the distribution, importance, knowledge of the life-cycle, laboratory breeding and morphology of the adult and immature stages of the genus *Culicoides*, with particular reference to existing knowledge of the morphology, distribution, life-cycle and habits of *C. impunctatus*, *C. obsoletus*, *C. odibilis*, *C. pallidicornis*, *C. cubitalis* and *C. chiopterus*.

2. An account is given of the rearing of *C. impunctatus* and *C. obsoletus* both in the

V

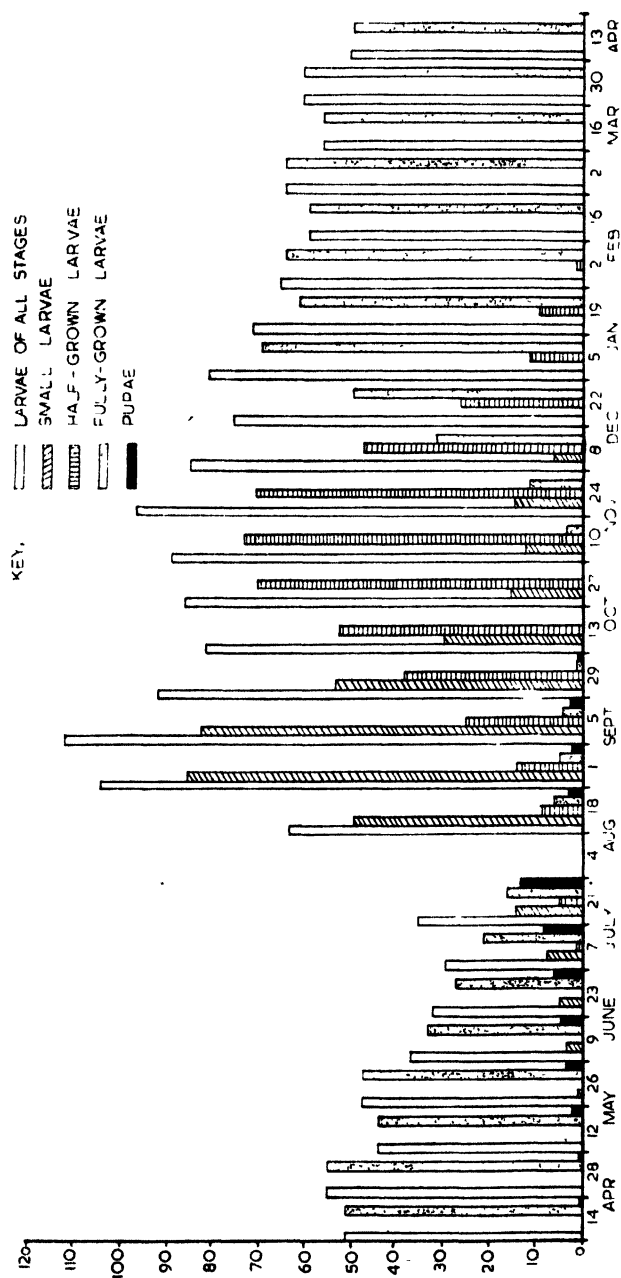
and of pupae recovered from each of six samples taken from plot 'B' every two-weekly period between April, April, 1946

Percentage of half-grown larvae in total recovered from six samples	Average no. of half-grown larvae recovered from each sample	Total no. of fully grown larvae recovered from six samples	Percentage of fully grown larvae in total recovered from six samples	Average no. of fully grown larvae recovered from each sample	Total no. of pupae recovered from six samples	Average no. of pupae recovered from each sample
0	0	309	100	51.5	2	0.33
0	0	331	100	55.16	3	0.5
0	0	264	100	44.0	12	2.0
0	0	282	98.95	47.0	18	3.0
0	0	201	92.2	33.5	27	4.5
0	0	165	86.0	27.5	34	5.66
1.7	0.5	128	72.7	21.33	50	8.33
14.3	5.0	96	45.7	16.0	78	13.0
—	—	—	—	—	—	—
13.5	8.5	34	9.0	5.66	19	3.16
13.83	14.36	27	4.34	4.5	12	2.0
22.8	25.3	24	3.6	4.0	14	2.3
41.3	37.8	7	1.3	1.17	10	1.66
63.7	51.8	0	0	0	0	0
82.5	70.5	0	0	0	0	0
82.6	72.8	19	3.6	3.16	0	0
73.2	70.16	67	11.7	11.16	0	0
55.9	47.16	186	36.8	31.0	0	0
34.4	25.83	296	65.6	49.3	0	0
13.7	11.0	416	86.3	69.3	0	0
12.9	9.16	370	87.1	61.66	0	0
2.3	1.5	385	97.7	64.16	0	0
0	0	356	100	59.3	0	0
0	0	384	100	64.0	0	0
0	0	333	100	55.5	0	0
0	0	360	100	60.0	0	0
0	0	299	100	49.8	0	0

laboratory and in the field, and of investigations into the habits and the incidence of the adults and immature stages of these two species throughout a complete year, as observed in Knowsley Park, Liverpool. Similar, though less complete, observations are presented of the life-cycle and habits of *C. odibilis*, *C. pallidicornis*, *C. cubitalis* and *C. chiopterus*.

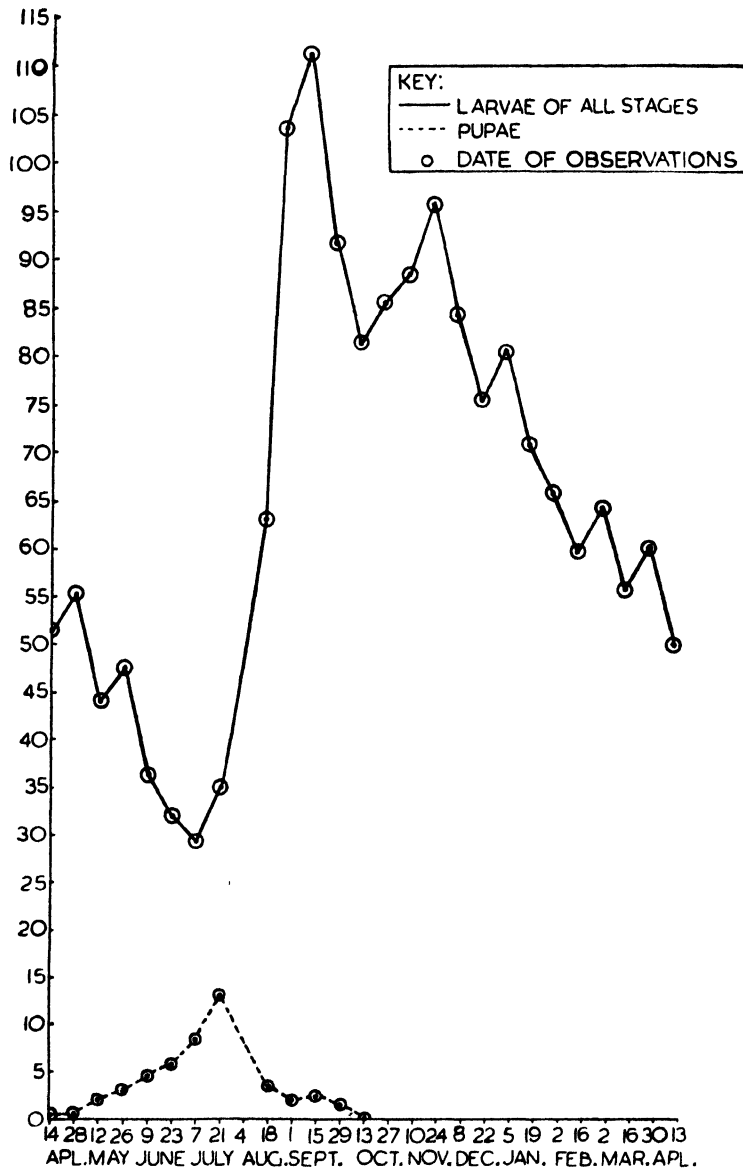
3. The techniques used for collecting the immature stages and adults of *Culicoides*, the handling and feeding of the adults, and the rearing of larvae under laboratory and field conditions are described.

4. *C. impunctatus* is the most abundant species in the Liverpool area, and by the application of Dyar's law it has been established that larvae of this species pass through



HISTOGRAM 2. The average numbers of larvae, of all stages, and the proportions of small, half-grown and fully grown larvae and of pupae recovered from each of six samples taken from plot 'B' every two-weekly period between April, 1945, and April, 1946

four instars. The winter is passed in the fourth larval stage, and these larvae begin to pupate at the end of April or the beginning of May, with a consequent drop in the



GRAPH 6. The average numbers of larvae, of all stages, and of pupae recovered from each of six samples taken from plot 'B' every two-weekly period between April, 1945, and April, 1946.

numbers of larvae recovered at that time. The last fourth stage larvae were recovered during the middle of July, which corresponded with the incidence of the last pupae

recovered. No larvae of this stage were recovered throughout the remaining part of the summer, and the next to be recorded were found at the beginning of November. During the intervening period the entire larval population consisted of second and third instar larvae. From May to July the population was composed almost entirely of second stage larvae, during August and most of September second and third stage larvae were about equally divided, while during the latter half of September and during October the population was almost entirely composed of third stage larvae. Owing to technical difficulties no first instar larvae were recovered. The first adult *C. impunctatus* were captured at the end of April, which corresponded with the finding of the first pupae of the 1945 season. The numbers of this species increased throughout May and reached a peak in June, the last specimens being caught in August. It would appear that the Knowsley Park strain of *C. impunctatus* normally passes through one generation per year, the life-cycle of a strain reared in the field occupying 11-12 months. In the laboratory the larval stage of *C. impunctatus* occupied up to seven months, but was usually of five months' duration. The pupal stage was about five days in length, and the eggs required about two weeks in which to hatch. About two weeks elapsed between the first blood-meal and oviposition, up to 65 eggs being deposited in one batch. Females were fertilized in the laboratory. This species is most abundant about half an hour before sunset and is active throughout the night and early morning. It would appear that it does not normally fly more than a few hundred yards from its breeding-ground. It has a marked preference for human blood (as opposed to *C. obsoletus*). The outstanding characteristic of all breeding-grounds of this species is peaty soil. An illustrated account is given of the morphology of the adult, egg, larva and pupa.

5. *C. obsoletus* is the next most common species to *C. impunctatus* in the Liverpool area. The larvae of this species began to pupate in April, and pupae were recovered throughout May and during September. The adults were captured during April, May and June, when they reached their peak. A second peak of abundance was recorded at the end of September. It would appear that the Knowsley Park strain of *C. obsoletus* normally passes through two generations per year, the life-cycle of a strain reared in the field occupying five months during the summer and nine months during the winter. In the laboratory the larval stage of *C. obsoletus* occupied up to five months, but was usually of three and a half months' duration. The pupal stage was about five days in length, and the eggs usually hatched within three days of being laid. Seventeen days elapsed between the first blood-meal and oviposition, up to 120 eggs being laid in one batch. Females were fertilized in the laboratory. An illustrated account is given of the morphology of the egg, larva and pupa.

6. The limited emergence periods of *C. odibilis*, *C. pallidicornis* and *C. cubitalis* indicate that these species pass through only one generation per year. An illustrated account is given of the morphology of the adults and their immature forms.

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# TRYPANOSOME COUNTS IN *TRYPANOSOMA CONGOLENSE* INFECTIONS

BY

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(Received for publication September 16th, 1946)

In all work on *Trypanosoma congolense* infections, the question arises of determining the correlation between the disease crises in the animal host and the number of trypanosomes present in the circulating blood, and for this purpose it is essential to have an accurate and consistent method of enumerating the trypanosomes.

Many workers express the numbers of parasites in relation to the erythrocytes, or as so many parasites per microscopic field, but this method does not give the trypanosome content of a unit volume of blood. Hornby and Bailey (1928) state that the counting of *T. congolense* is very easy, provided that the counting-chamber does not exceed 0.02 mm. in depth. They, however, were working with trypanosomes separated from heavily infected rat blood; moreover, such a counting-chamber was not available when the present work was done. Yorke, Adams and Murgatroyd (1929) described a method of counting *T. equiperdum* in an ordinary counting-chamber; but this was tried without success. Further attempts, using varying dilutions in a standard red-cell diluting pipette and citrate-Ringer-glucose, Hayem's fluid, and 0.1 per cent. formol saline as the diluent, were all unsuccessful.

A method based on comparison with the number of leucocytes present in a stained thick drop was then tried, and this was found to give consistent results when counts were made on duplicate slides.

## METHOD

Oxalated samples of venous blood were taken from infected bovines. By means of a Neubauer counting-chamber and a dilution of 1/20 with 3 per cent. acetic acid, all leucocytes present over the 9 sq. mm. ruling were counted and the total number per c.mm. was calculated. Blood from the collecting-needle was then used to make thick smears.

The thick smears were first dipped for one or two seconds into a 0.1 per cent. aqueous solution of azur II, and were then stained for 30 minutes in azur II-eosin mixture (Laws, 1931).

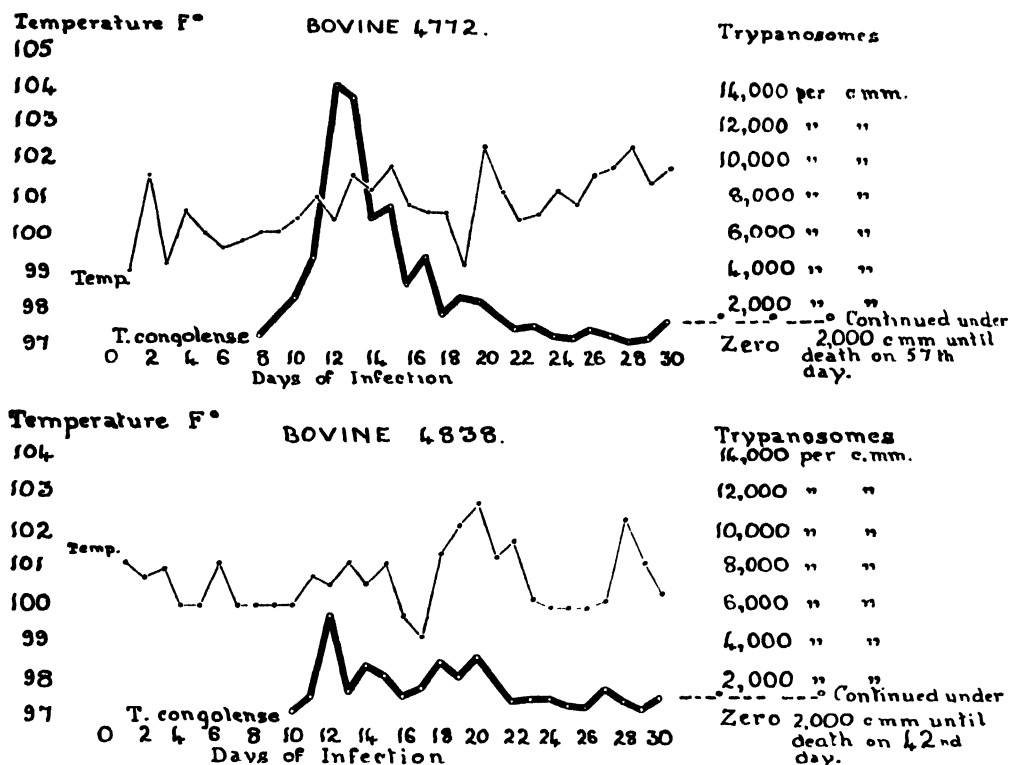
This method preserves the morphology of the leucocytes, and trypanosomes are well stained. To facilitate counting and to avoid eyestrain, a stop was placed in the field of the eyepiece, consisting of a circle of black paper with a slot approximately 8 mm. wide cut out across the centre line, thus masking the top and bottom portions of the field.

The ratio of trypanosomes to 500 or 1,000 leucocytes was then found, and from the figure obtained the number of trypanosomes per c.mm. was calculated.

Using the method described, trypanosome counts were made daily throughout the course of *T. congolense* infection in two Ankole bovines. These bovines, not previously infected with trypanosomiasis, were inoculated with infected mouse blood of the

'Kikaya' strain of *T. congolense*, which had been maintained by 42 serial passages in mice (average survival-period 14 days). The strain was pathogenic to bovines, for five out of six cattle inoculated with blood of the same passage died within two months of infection.

The counts obtained are illustrated graphically below.



In view of the suggestion made by Richardson (1946) that there is a rhythmic development cycle in *T. congolense* and *T. vivax* infections, and that cure may depend on the administration of a drug at the right point in the cycle, it is desired to repeat this work on a larger scale.

This note is published in the hope that other workers in this field will come forward with an accurate but less tedious technique.

**ACKNOWLEDGEMENTS.**—The writer is indebted to the Director of Veterinary Services, Uganda, for permission to publish this note.

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# SODIUM STIBOGLUCONATE IN THE TREATMENT OF KALA-AZAR :

## REPORT ON THE TREATMENT OF EIGHT CASES AND THE APPEARANCE OF PROBABLE DRUG REACTIONS

BY

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*(Received for publication February 15th, 1947)*

Sodium stibogluconate is a pentavalent antimonial prepared by Messrs. Burroughs Wellcome and Co. for the treatment of visceral leishmaniasis. A report of its successful use in the treatment of kala-azar in the Sudan appears elsewhere in this journal (Kirk and Sati, 1947). We have used the drug in eight cases of proved kala-azar. Three cases completed the full course of treatment without incident. Five cases developed rigors associated with high fever during treatment; one of these cases died. It was possible to re-examine six patients 3-9 months after treatment. All had apparently recovered from the disease.

### CLINICAL MATERIAL

Our patients were Servicemen who had served either in India or in the Mediterranean littoral or in both. The clinical histories revealed an illness lasting from a few weeks to eight months prior to admission to the Tropical Diseases Centre, Smithdown Road Hospital, Liverpool, and characterized by splenic enlargement, fever and sweating—the latter especially at night. Five cases were diagnosed as kala-azar before admission. In all, the leucocyte count was low on admission, ranging from 1,000 to 5,400 cells per c.mm. of blood. The sternal bone-marrow smear or culture or both were positive for *L. donovani* in seven cases; in the eighth case (B. A.) *L. donovani* was found in the smear and in cultures from fluid obtained by splenic puncture.

Some of the clinical aspects of the eight cases are set out in Table I. Full details may be obtained on request from the editors of these *Annals*.

### TREATMENT

A dilute solution of the drug (containing the equivalent of 20 mgm. antimony per c.cm.) was injected intravenously in all cases. The drug was administered in doses of 12 c.cm. (equivalent to 240 mgm. antimony) twice daily, at 8-12 hour intervals. It was intended to give 12 c.cm. twice daily for 10 days as a standard course, but, as will be

seen from Table I, the full course was administered in three cases only. The fatal case received 12 injections. One case (G. P.) received two courses of treatment (see Table I), the first without incident, the second with a febrile reaction.

### RESULTS OF TREATMENT

I. Three cases received the full course of treatment without incident. In two (J. C. and H. G.) the temperature was normal by the fifth day of treatment, and in the third (H. F.), in whom the fever was not marked at the commencement of treatment, the temperature rose above normal only once after the fourth day of treatment. There was a further unexplained rise of temperature in J. C., starting on the eighth day of treatment and lasting for five days, but after this episode this patient improved rapidly. Progress was excellent in all three cases.

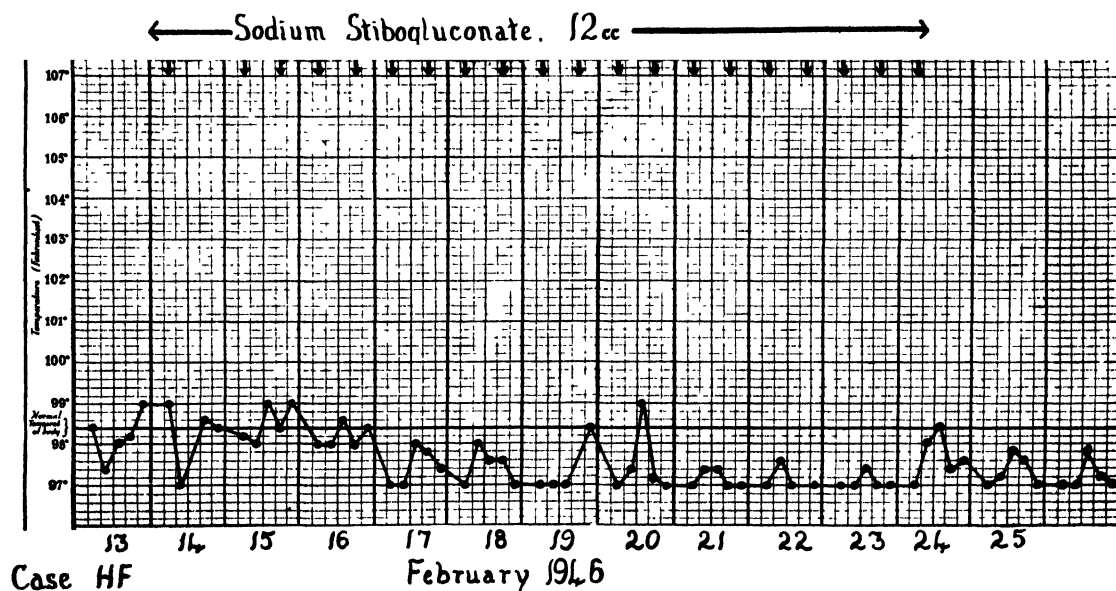


CHART 1. Temperature and dosage record of case H.F. Each arrow represents an intravenous injection of 12 c.cm. sodium stibogluconate.

The case of H. F. may be quoted as an example of this group of patients. This case is of particular interest, because he was given a full course of the batch of drug administered a month later to the fatal case.

#### CASE H.F. Aged 39. (See chart 1)

Served from 1942 to April, 1945, in India, including Assam. He kept well until November, 1944, when he developed fever, sweating and a large spleen. He was diagnosed as kala-azar, although *L. donovani* was not found in the sternal bone marrow. He was treated in July, 1945, with a course of neostam and subsequently for 'persistent anaemia and leucopenia.'

1.2.46. On admission to the Centre he stated that he felt well but had no energy. He felt slightly feverish at times and sweated a good deal. He looked anaemic. The spleen was palpable two fingers' breadth below the costal margin. Temperature 98.2° F. Blood: erythrocytes, 2.7 million per c.mm.; haemoglobin, 55 per cent.; leucocytes, 2,300 per c.mm.

TABLE I  
Details of eight cases of kala-azar treated with sodium stibogluconate

Case	Service	Length of illness on admission	Spleen on admission (fingers' breadth below costal margin)	Leucocytes on admission (per c.mm.)	Diagnosis	Treatment	Reaction	Follow-up
1. J.R., aged 24 (English)	Royal Navy India ; East and North Africa ; Italy ; Spain ; Portugal.	7 months. Admitted 3.4.45	+ 8	1,200	<i>Sternal bone marrow</i> : Smear : L.D. bodies positive. Culture : positive.	(i) 4.4.45 : 100 mgm. <i>urea stibamine</i> . (ii) 7.4.45-24.4.45 : 1,200 mgm. <i>carbo-stibamide</i> . (iii) <i>sodium stibogluconate</i> (Batch No. AN99760) 12 c.cm. twice daily. Commenced 1.5.45. Ceased 7.5.45.	5.5.45 : <i>Rigor</i> commencing $\frac{1}{2}$ hour after evening dose. Temperature returned to normal in 24 hours.	5.2.46 : 'No evidence of kala-azar.'
2. J.C., aged 26 (English)	Merchant Navy Russia ; Iraq ; Sicily ; Italy.	5 months. Admitted 9.6.45	Enlarged to umbilicus	5,000	<i>Sternal bone marrow</i> : Smear : L.D. bodies positive.	<i>Sodium stibogluconate</i> (Batch No. AN99760) 12 c.cm. i.v. twice daily. Commenced 13.6.45. Ceased 22.6.45.	No reaction.	18.10.45 : <i>Sternal bone marrow</i> : Smears and cultures negative for L.D. bodies.
3. A.C., aged 33 (Italian)	Italian prisoner-of-war Served in N. Italy and S. Germany only.	3 months. Admitted 7.9.45	+ 5	1,100	<i>Sternal bone marrow</i> : Smear : L.D. bodies positive.	<i>Sodium stibogluconate</i> (Batch No. AN99760) 12 c.cm. i.v. twice daily. Commenced 8.9.45. Ceased 14.9.45.	14.9.45 : <i>Rigor</i> commencing $\frac{1}{2}$ hour after evening dose. Temperature 105° F. Returned to normal in 36 hours.	11.12.45 : <i>Sternal bone marrow</i> : Smears and cultures negative for L.D. bodies. 22.1.46 : Serial blood cultures negative for L.D. bodies.

aged 27 (English)	1942-45 : India, including Cal- cutta and Kara- chi. 1945 : Singapore.	mately 1 month. Admitted 15.11.45			row : Smear : negative. Cultures : posi- tive for L.D. bodies.	<i>Sodium stibogluconate</i> (Batch No. AN99760) 12 c.cm. i.v. twice daily. Commenced 19.11.45. Ceased 29.11.45.	No reaction.	12.2.46 : Sternal bone mar- row : Smears and cul- tures negative for L.D. bodies.
5. B.A., middle- aged (Indian)	Merchant Navy Calcutta to Bir- kenhead.	Acutely ill for 1 week. Admitted 4.12.45	+ 3	4,600	<i>Splenic puncture</i> : Smears and cul- tures positive for L.D. bodies.	<i>Sodium stibogluconate</i> (Batch No. AN99760) 12 c.cm. twice daily. Commenced 5.12.45. Ceased 13.12.45.	12.12.45 : Rigor commenc- ing after even- ing dose. Temperature 103.2° F. Normal in 24 hours.	None.
6. G.P., aged 34 (English)	Army 1943-April, 1944: India, including Assam. April, 1944 - March, 1945 : Ceylon.	6 months. Admitted 14.11.45	+ 2	1,000	<i>Sternal bone mar- row</i> : Smears and cul- tures positive for L.D. bodies.	<i>Sodium stibogluconate</i> (Batch No. AN99760) (a) 16.11.45 : 12 c.cm. twice daily for 6 days. (b) 18.12.45 : 6 c.cm. twice daily for 3 days ; then 12 c.cm. twice daily for 3 days. Commenced 18.12.45. Ceased 24.12.45.	(a) None. (b) 24.12.45 : Rigor commenc- ing ½ hour after morning dose. Temperature 103° F. ; nor- mal in 24 hours.	16.3.46 : Sternal bone mar- row : Smears and cul- tures negative for L.D. bodies. Blood cultures : Negative.
7. H.F., aged 39 (English)	Army 1942-45 : India, including Assam.	3 months. Admitted 1.2.46	+ 2	2,300	<i>Sternal bone mar- row</i> : Smears : positive for L.D. bodies. Cultures : neg- ative.	<i>Sodium stibogluconate</i> (Batch No. TH26504) 12 c.cm. i.v. twice daily. Commenced 14.2.46. Ceased 24.2.46.	No reaction.	2.7.46 : Sternal bone mar- row : Smears and cul- tures negative for L.D. bodies.
8. E.L., aged 30 (negro)	Army 1942-45 : Italy and Egypt.	8 months. Admitted 2.11.45	+ 1	5,400	<i>Sternal bone mar- row</i> : 26.11.45 : smears and cultures neg- ative. 26.2.46 : Smears positive for L.D. bodies. 22.1.46 : Serial blood cultures	<i>Sodium stibogluconate</i> (Batch No. TH26504) 12 c.cm. i.v. twice daily. Commenced 5.3.46. Ceased 10.3.46.	10.3.46 : Rigor commenc- ing ½ hour after evening dose. Temperature 107° F.	11.3.46. Died.

5.2.46. Sternal bone marrow : scanty L.D. bodies seen. Cultures negative for *L. donovani* after 18 days. Formol-gel test strongly positive after two hours.

14.2.46. A course of intravenous sodium stibogluconate 12 c.cm. twice daily was started and continued without incident (except for a symptomless rise of temperature to 99° F. on 20.2.46) until 24.2.46.

28.2.46. Blood : erythrocytes, 4.7 million per c.mm. ; haemoglobin, 79 per cent. ; leucocytes, 4,000 per c.mm.

14.3.46. Discharged.

27.6.46. Returned to Centre for test of cure. Felt very fit. Spleen and liver not palpable.

2.7.46. Sternal bone marrow : no *L. donovani* seen. Cultures negative after 18 days.

3.7.46. Blood : haemoglobin, 91 per cent. ; leucocytes, 7,250 per c.mm.

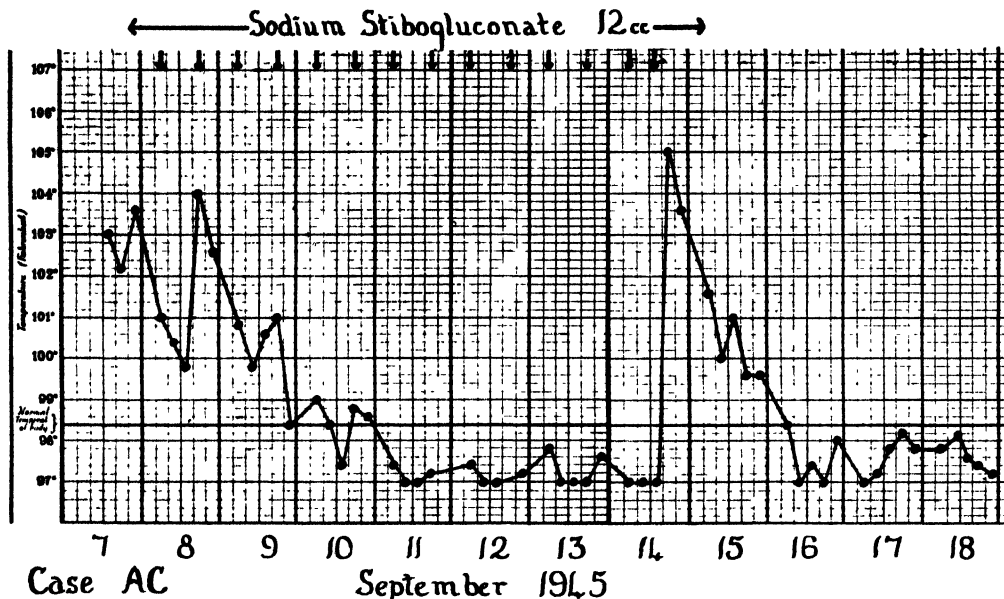


CHART 2. Temperature and dosage record of case A.C. Each arrow represents an intravenous injection of 12 c.cm. sodium stibogluconate.

II. In five cases a rapid rise of temperature, associated with a severe rigor, occurred during the course of treatment, in one case after the 10th dose, in three after the 12th dose, and in one after the 14th dose. In every case the rigor and rise of temperature commenced without warning about half an hour after the intravenous injection of the drug. One case (E. L.) was fatal ; in the others the temperature returned to normal in 24-48 hours. In the surviving cases clinical progress was maintained after the subsidence of the temperature to normal.

The development of rigor and fever, followed by recovery, is illustrated in the following case.

#### CASE A.C. Aged 33. (See chart 2)

An Italian prisoner-of-war who had served in northern Italy and southern Germany. He was brought to England in May, 1945, and soon afterwards fell ill, complaining of abdominal pain and shivering attacks, which occurred off and on for three months until admission to the Tropical Centre.

7.9.45. On admission he complained of abdominal pain below the right and left rib margins. The spleen was palpable, hard and tender, and enlarged to five fingers' breadth below the costal margin. The temperature on admission was 103° F. ; pulse-rate about 100.

8.9.45. Blood : erythrocytes, 2.7 million per c.mm. ; haemoglobin, 34 per cent. ; leucocytes, 1,100 per c.mm., with some increase in lymphocytes. Formol-gel test : serum opaque and solid within two minutes. Sternal bone marrow : *L. donovani* seen in smear. Commenced a course of intravenous sodium stibogluconate 12 c.cm. twice daily for 10 days.

11.9.45. Much better. Temperature normal.

14.9.45. Morning dose given without incident. Half an hour after the evening injection a severe rigor developed. The temperature rose rapidly to 105° F., and the pulse-rate to 130. The patient complained of severe abdominal pain, mostly in the liver region, and of diarrhoea. The temperature fell steadily, and returned to normal after 36 hours.

20.9.45. Spleen now palpable only one finger's breadth below the costal margin. A severe outbreak of herpes appeared round the mouth and lips. There was oedema of both lips, and the submental glands were palpable and tender. The oedema disappeared in 48 hours and the herpes slowly cleared up.

4.10.45. Spleen now just palpable below costal margin.

11.10.45. Leucocytes, 5,500 per c.mm.

15.10.45. Formol-gel test negative after two hours.

The patient rapidly improved and was sent to the convalescent depot.

11.12.45. Returned for test of cure. Blood : haemoglobin, 101 per cent. ; leucocytes, 6,100 per c.mm. Sternal bone marrow : smears and cultures negative for *L. donovani*.

22.1.46. Serial blood cultures negative for *L. donovani*.

15.2.46. The patient had an attack of severe biliary colic. He was examined by the consulting surgeon and a laparotomy was performed on 1.3.46. A diagnosis of cholecystitis and chronic appendicitis was made ; the appendix was removed. 'Rest of abdominal contents normal.'

8.4.46. Discharged.

### III. The fatal case.

#### CASE E.L. Aged 30. (See chart 3)

This patient, a negro, was born in British Guiana and served in southern Italy in July, 1944, and in Egypt from October, 1944. In April, 1945, he began to have heavy sweating attacks and developed persistent fever. *L. donovani* was eventually found in the sternal bone marrow. A course of solustibosan produced little improvement. This was followed by a course of stilbamidine, which checked the fever and sweating. The latter treatment was finished in August, 1945, and the patient was evacuated to England.

2.11.45. On admission to the Centre the patient complained of feeling weak, but he was not feverish and said that he had not been sweating at night. The temperature and pulse were normal. The spleen was palpable two fingers' breadth below the costal margin. The liver edge was palpable and slightly tender. There was some general enlargement and tenderness of the lymph nodes in the axillae. The air-entry at the right lung base was poor. Blood : haemoglobin, 90 per cent. ; leucocytes, 5,400 per c.mm. Formol-gel test : no opacity or coagulation after 60 minutes.

26.11.45. Sternal bone marrow : no *L. donovani* seen. Faeces : cysts of *E. histolytica* present.

The patient was given the standard three-weeks course of treatment for the *E. histolytica* infection (auremetine gr. 1 four times a day on alternate days ; quinoxyl enemata and stovarsol on days on which auremetine was not received).

After treatment he was sent to a convalescent depot, and on return felt much better and had gained 3 lb. in weight. Cysts of *E. histolytica* were not found again in the faeces, but hookworm eggs were observed from time to time, though never in large numbers.

A fortnight after his return from the convalescent depot an evening rise of temperature to 99° F. was noted on three consecutive days. On the fourth day the patient had a mild rigor, the temperature rising to 102.2° F. He complained of pain and tenderness in the left upper abdomen and the spleen was easily palpable and tender. No malaria parasites or *L. donovani* were seen in the peripheral blood. The patient rapidly improved and was later allowed to go on leave.

On returning from leave he was found to have acquired a Neisserian urethritis. His leucocyte count was 6,000 per c.mm., and the spleen was still palpable one finger's breadth below the costal margin. The temperature oscillated between 100° and 97° F.

A course of sulphathiazole was administered (sulphathiazole 3 gm. stat. ; 1.5 gm. four-hourly for two days ; 1.5 gm. six-hourly for two days ; 1.0 gm. six-hourly for two days). The urethral discharge ceased, and the temperature returned to normal.

22.1.46. Formol-gel test : opacity and coagulation after two hours. Serial blood cultures negative for *L. donovani*. Blood : erythrocytes, 4.6 million per c.mm. ; haemoglobin, 71 per cent. ; leucocytes, 6,400 per c.mm.



22.2.46. Sternal bone marrow : *L. donovani* present in smear.

5.3.46. A course of sodium stibogluconate 12 c.cm. intravenously twice daily commenced.

For the fortnight previous to this treatment the patient had been feeling better, but the temperature had occasionally been as high as 101° F. in the evening. The spleen had not become appreciably larger since admission.

During treatment the evening temperature rose to 99.4° F. on the third day and to 100.8° F. on the fifth. These peaks of fever were of short duration and were accompanied by no unusual symptoms.

10.3.46. The morning temperature was 98.4° F. The injection of the drug in the morning was given without incident. The administration of the afternoon dose, however, was followed within half an hour by a very severe rigor, during which the temperature rose to 107° F. and the pulse-rate to 160. The temperature was reduced to 104° F. by tepid sponging, and had fallen to 102° 12 hours later. From this point it continued to fall rapidly, and by midday was normal; pulse-rate about 120.

At 11 a.m. the patient vomited two pints of bile-stained fluid containing altered blood. From the time of the development of the high fever the patient was cyanosed and shocked (B.P. 70/50 at 12 a.m.), and was treated accordingly. At 2 p.m., i.e., 21 hours after the last dose of the drug was injected, air-hunger developed suddenly and he collapsed and died.

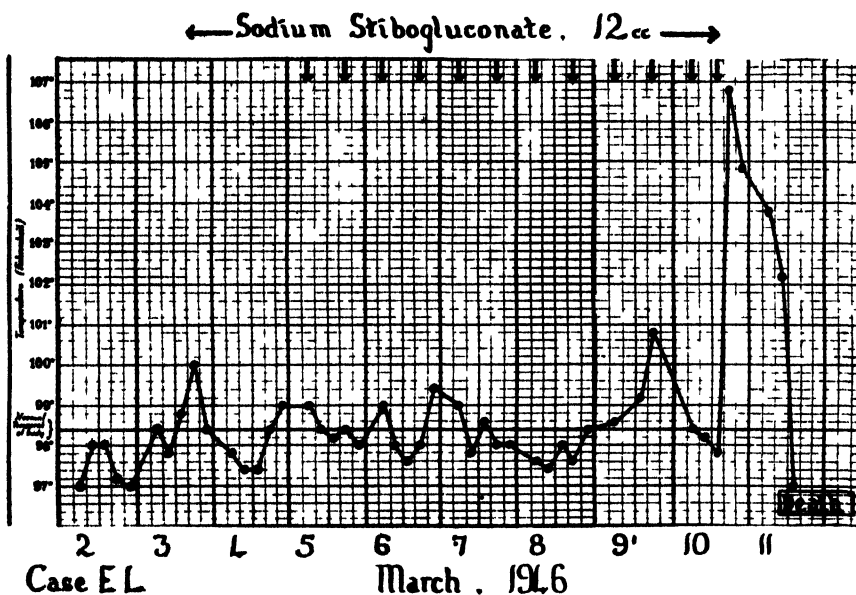


CHART 3. Temperature and dosage record of case E.L. Each arrow represents an intravenous injection of 12 c.cm. sodium stibogluconate.

#### Autopsy Report

The body was that of a well-developed coloured man, corresponding to a stated age of 30 years. Generalized rigor mortis was present.

There were no abnormalities of skull or brain.

There were fibrinous adhesions of the pleura on the right side only, and the pleural surface of both lungs showed scattered linear haemorrhages, with a continuous line of haemorrhage from capillaries in the position stated. There was no other abnormality of the lungs.

The myocardium was rather pale and suggested a state of early brown atrophy.

The stomach contained a moderate amount of colourless fluid, in which were suspended flecks of altered blood, and the duodenum, which was very markedly congested, appeared to have been the source of this blood. There was no abnormality of the jejunum or ileum.

The large intestines exhibited occasional fairly congested islands of mucosa, and at one point on the ascending colon was what appeared to be a relapsing amoebic ulcer. The spleen was firm and approximately four times enlarged.

The liver was pale and the cut surface suggested areas of focal necrosis.

The kidneys also were pale, and showed some congestion of the vessels of both cortex and medulla.

The blood-vessels of both cortex and medulla were organically normal, but wherever there was loose tissue there had been exudation from the capillaries into it.

*Cause of Death.* This man was suffering from kala-azar and amoebic dysentery, and died of acute circulatory failure during the course of his treatment.

### *Histological Report*

*Cardiac Muscle.* There are brown granular deposits around the poles of some nuclei. There is no fragmentation or vacuolization of fatty degeneration of the cytoplasm. The nuclei appear normal. There is thus no evidence of the myocardial damage reported elsewhere in antimony poisoning (Bradley and Fredrick, 1941).

*Liver.* The cells are swollen. There is wide-spread granular degeneration, with some fragmentation of the cytoplasm. There is some scattered light yellowish-brown pigment in the cells. Occasional nuclei are pyknotic, but the nuclei in most cells appear normal. In some areas, however, there is extensive cytoplasmic degeneration, with necrosis of occasional cells. The changes in the cells are more obvious in the centre of the lobules than in the region of the portal systems. The sinuses contain few red cells, and appear in places to be obstructed by the swollen liver and Kupffer cells. The central veins are wide and empty of blood. The Kupffer cells are greatly enlarged, and some of them contain Leishman-Donovan bodies. Cell debris and red blood-corpuscles are occasionally present in the cytoplasm of Kupffer cells. There is some degree of infiltration of the tissue with small round cells and occasional polymorphs.

TABLE II  
Antimony content of organs of case E.L. (estimated by Dr. Tudor Jones)

Specimen	Estimated weight of organ in gm.	Antimony in organs		Approximate percentage rendered antimony in organs
		Mgm. per gm.	Total, in mgm.	
Formaldehyde in which organs were preserved ... ..	—	—	—	—
Liver ... ..	1,500	0.191	286	73
Spleen ... ..	500	0.161	80	21
Kidneys ... ..	300	0.081	24	6
Lung ... ..	—	—	—	—
Voluntary muscle ... ..	—	—	—	—
Heart-muscle ... ..	—	—	—	—
Bile ... ..	—	0.02 mgm./l.	—	—

*Spleen.* The sinuses are greatly congested. Occasional Leishman-Donovan bodies are present within the cytoplasm of large mononuclear macrophages.

*Kidneys.* There is scattered congestion of the glomerular capillaries. Bowman's capsules are normal in size and free from debris. There is some granular degeneration of the epithelium of the convoluted tubules, some of which contain shed epithelial debris. In scattered areas there is intense congestion of the medulla and, in places, haemorrhage into the tubules.

*Lungs.* Congestion and patchy oedema, but no histological evidence of haemorrhage into interstitial tissue.

*Notes on the Antimony Content of Specimens of the Organs Taken at Autopsy.\** The organs examined contained something more than 350 mgm. antimony, out of a total amount of 2,880 mgm. administered; 240 mgm. was the last dose, which was not excreted. This shows that the previous excretion of antimony had been satisfactory, and that death cannot have been caused by accumulation of antimony.

The antimony content of the organs submitted to Mr. Goodwin for analysis is shown in Table II.

\* Supplied by kindness of Mr. L. G. Goodwin, of the Wellcome Laboratories of Tropical Medicine, London.

IV. It was possible to follow up the histories of six patients subsequent to treatment. The results are summarized in Table I. It will be seen from the table that, as far as the follow-up could be carried out, cure of the disease had been effected in five cases. In the sixth case details of the follow-up are unfortunately missing from the case-notes, beyond the statement quoted that 'there was no evidence of kala-azar.' It was not possible to trace the subsequent history of the Indian patient (B. A.).

### DISCUSSION

In six of the eight cases treated with sodium stibogluconate recovery from the disease occurred, so far as could be judged over the period of observation. The drug can therefore be regarded as an active therapeutic agent in kala-azar.

In five cases, however, after the 10th, 12th or 14th doses severe rigor occurred, associated with high fever. The appearance of this reaction was independent of the severity of the case before treatment and of the geographical origin of the disease. We could see no relation between the clinical condition of the patient and the appearance of the reaction. We consider, therefore, that the latter was probably attributable to the drug.

TABLE III  
Amount of antimony injected during course of treatment

Case	No. of doses		Antimony, in mgm.	Reaction
	12 c.cm.	6 c.cm.		
J.R.	14	—	3,360	+
J.C.	20	—	4,800	O
A.C.	14	—	3,360	+
H.G.	20	—	4,800	O
B.A.	15	—	3,600	+
G.P., i	12	—	2,880	O
" ii	5	7	2,280	+
H.F.	20	—	4,880	O
E.L.	12	—	2,880	+

There is no evidence to suggest that the antimony in the drug was directly responsible. The amount of antimony which our patients received is shown in Table III. It will be seen that there is little relation between the size of the dose injected and the development of the reaction, except that the patients who did not react received heavier doses of antimony than those who did. In the case of G. P., who reacted only after the second course of the drug, more antimony was administered in the first treatment than in the second. In the fatal case the organs examined contained about the equivalent of two doses of the drug; half of this could be accounted for by the last dose given, which was not excreted. As Mr. Goodwin reports, this finding indicates that previous excretion of antimony must have been satisfactory, and that death cannot therefore be regarded as the result of accumulation of the metal in the tissues. Further, the histological findings in this case did not indicate poisoning by antimony. Finally, in two cases the injections were continued after the reaction, with no ill effects.

It is possible that the organic basis of the sodium stibogluconate might have played a part in the development of the reaction. In all cases rigor commenced about half an

hour after the injection. This suggests that some sensitization factor might have been involved. It is difficult, however, to see how such sensitization could be produced by a therapeutic régime in which the drug was administered twice daily. Reactions of an anaphylactic nature have been reported during therapy with pentavalent antimonial compounds. These are rare and have 'not been observed when daily injections are given but . . . in a few cases in which wider spacing of the injections was adopted' (Napier; 1938).

There is no direct evidence that the reactions which we observed were of an anaphylactic nature, but the continuation of the treatment without ill effect following the reaction would be consistent with this. Sensitization by previous use of sodium stibogluconate or solustibosan is conceivably possible in patients G. P. and E. L., and other organic antimonials which might have produced sensitization were used in case J. R.

Mild reactions, marked by transient pyrexia and rigors, have recently been reported in two out of six cases of kala-azar treated with a new pentavalent antimonial compound '2.168 R.P.' (Durand, Benmussa and Caruana, 1946). This drug was administered intramuscularly every two or three days, and the reactions occurred after the third and fifth injections in one case and after the second and sixth injections in the other. Four other cases were treated with larger doses of the drug without ill effect. In discussing their results, the authors write: 'on note, à une ou deux reprises, le soir d'une injection et dans les premières injections, des phénomènes de malaise général avec frisson et légère élévation thermique dont il est difficile de dire s'il s'agit là d'une conséquence de la piqûre ou d'une manifestation leishmanienne.' The latter possibility should perhaps also be considered in our cases.

It is unlikely that the sodium stibogluconate we used was chemically faulty, since patients H. F. and E. L. received a batch of drug different from that which was used in patients J. R., A. C., B. A. and G. P. Moreover, the patients H. F. and E. L. received the same batch of drug within a month of each other, and the former did not react, whereas the latter died. The following notes on the toxicity of the batch of drug used in these two patients have been supplied by Mr. Goodwin. 'The toxicity of the batch is much less than that of our standard based upon a sample of German solustibosan manufactured before 1939, and about the same as the toxicity of a later German batch recently "liberated" from Leverkusen.' The pH of the batch returned by us to Mr. Goodwin following the death of E. L. was 5.3. Mr. Goodwin reports that the pH in the solution supplied was adjusted to 5.5, since acid solutions are much less toxic than neutral or alkaline ones.

### SUMMARY

Eight cases of kala-azar were treated with sodium stibogluconate by intravenous injection of 12 c.cm. of a weak solution twice daily. In five patients rigor, associated with high fever, developed half an hour after the injection of the 10th or 14th dose of the drug. One patient died of vascular failure 21 hours subsequent to the onset of the rigor.

Six patients were followed up after treatment for periods ranging from three to nine months, and satisfactory progress was reported in all. In five of these patients *L. donovani* were no longer present in the sternal bone marrow at the last examination. As far as our observations go, therefore, the drug appears to have effected a cure.

**ACKNOWLEDGEMENTS.**—We are very grateful to Mr. L. G. Goodwin for arranging for the analysis of the organs from case E.L. and for reporting on the toxicity of the batch of drug used in this case. We acknowledge with thanks the help of Sister McNair and members of the staff of the Tropical Diseases Centre, Smithdown Road Hospital, Liverpool. We also acknowledge help and advice from Dr. C. M. Wenyon, of the Wellcome Institute, and from Dr. J. P. Steel, Medical Superintendent of Smithdown Road Hospital.

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# THE PART PLAYED BY THE FAECES AND VOMIT-DROP IN THE TRANSMISSION OF *ENTAMOEBA HISTOLYTICA* BY *MUSCA DOMESTICA*

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## INTRODUCTION

The important rôle played by flies of the genus *Musca* in the transmission of both bacillary and amoebic dysentery is now well recognized, and it is generally agreed that, although flies belonging to other genera may sometimes be involved, the latter's share in spreading these diseases is relatively insignificant.

A survey of the literature concerning bacillary dysentery shows that numerous investigators have proved the causal organism to be conveyed by the fly, not only in its faeces and, to a lesser extent, on its feet and body, but also—and probably most frequently—in its vomit-drop. As early as 1903 Smith, in his reports on municipal sewerage, discussed the possible dissemination of dysentery bacilli by house-flies, and similar views were put forward by Bergey (1907). These conjectures received strong support from the work of Auché (1906), who had shown that laboratory-bred flies, when fed on cultures of dysentery bacilli, subsequently passed these organisms in a viable condition in their faeces, and this conception was proved by the experimental work of Orton and Dodd (1910), who recovered *B. dysenteriae* (Shiga) from flies caught in the Worcester State Hospital, Massachusetts. Finally, Bahr (1914) isolated *B. dysenteriae* (Shiga) from the intestinal tracts of flies caught in the vicinity of patients suffering from the disease, and showed that these organisms persisted in the flies up to five days after their ingestion. These observations on flies, made under natural conditions, were confirmatory of Bahr's (1912) own results obtained under laboratory conditions in Fiji in 1910.

In the case of amoebic dysentery, as in that of bacillary dysentery, various early workers suggested that house-flies might play a part in the transmission of the disease, but, contrary to the success attending first attempts to prove the importance of the house-fly as a vector of bacillary dysentery, negative or inconclusive results sometimes followed experiments with *E. histolytica*. Thus Kuenen and Swellengrebel (1913), working in Java, introduced flies (*Musca domestica*) into a series of bottles containing faeces rich in *E. histolytica* cysts, the flies being subsequently examined for the presence of cysts both on the surface of the body and in the gut. As might be expected from such a technique, cysts were recovered from both sites, but the majority recovered from the gut were considered to be non-viable, as judged by the eosin test, and the authors concluded that the part played by the house-fly in the transmission of amoebic dysentery was negligible. Jausion and Dekester (1923) fed a number of species of flies, including *M. domestica*, on faeces containing cysts of *E. histolytica*. The flies were then killed at various intervals

\* Working under a grant from Imperial Chemical Industries Limited.

after feeding, washed in serum, and the centrifuged deposit and the guts examined for cysts. The results in both instances were negative, nor did the injection of this material into the rectum of a kitten result in dysentery.

These few failures were probably due to faulty technique, and in any case are insignificant when compared with the more numerous successful results, obtained both with flies found naturally infected and with those infected in the laboratory, which have been recorded by other workers. Among the earliest investigators who obtained positive results were Thomson and Thomson (1916), who fed cysts of *E. histolytica* to flies collected in the vicinity of army camps in Egypt. After 12 hours the flies were dissected and a large number of cysts were found in the intestines. Two years later a careful series of experiments was carried out by Roubaud (1918), who fed *M. domestica* on faeces containing *E. histolytica* cysts, and subsequently found cysts in the gut and in the faeces of flies up to 50 hours after the infective feed. These cysts appeared to be normal in every respect, and, since they resisted eosin staining, Roubaud considered that they were viable and capable of producing infection if fed to a human host. He therefore regarded the house-fly as a potential vector, but, since he considered the cysts to be susceptible to drying, he believed that infection could only occur if the faeces were deposited on a liquid or very humid medium. A more comprehensive study of the protozoa found in the intestines of wild flies, both before and after feeding on faeces containing *E. histolytica* cysts, was made by Wenyon and O'Connor (1917) during an extensive investigation of the human intestinal protozoa occurring in Mesopotamia. These authors carried out three series of experiments. In the first series, house-flies were fed on faeces containing *E. histolytica* cysts and were dissected at intervals up to 72 hours after feeding. Of 44 flies thus fed and dissected, 19 had no faeces in the gut, and, of the remaining 25 flies which had faeces in the gut, *E. histolytica* cysts were found in eight. In the second series, 17 flies were fed on faeces containing *E. histolytica* cysts, a proportion of which were found in all the faecal drops subsequently deposited. In the third series, 229 house-flies were caught and put into separate containers; 608 of the faecal drops deposited by these flies were examined for *E. histolytica* cysts, which were found in the faeces passed by five of the flies. An extensive examination of flies caught in the field was carried out by Buxton (1920), who dissected *M. domestica* occurring near habitations in Mesopotamia and found that of 1,027 flies caught and examined 60 per cent. contained human faeces. Seven hundred and thirty-three of these flies were dissected and 0.5 per cent. were found to contain *E. histolytica* cysts in their intestines. Buxton also examined the vomit-drops passed by these flies but failed to recover cysts in any of them. Further confirmation of the part played by the house-fly in the transmission of cysts was given by Root (1921), who, while working in America, fed laboratory-bred *M. domestica* on faeces containing large numbers of *E. histolytica* cysts, and later found viable cysts, as judged by the neutral red test, in their faeces. Frye and Meleney (1932) carried out a survey of the human intestinal protozoa occurring in a rural community in Tennessee, which in its extensiveness resembled that undertaken by Wenyon and O'Connor in Mesopotamia, and which similarly included observations on the part played by house-flies in the dissemination of amoebic dysentery. These authors collected house-flies both inside human habitations and at varying distances from the houses. The individual dissection of 103 flies caught inside houses yielded negative results, but mass examinations proved more successful. In the first series of mass examinations, 18 collections of flies were made out of doors and a similar number indoors,

each collection being contained in a separate bottle. After 24 hours a fixative was added to each collection, and the fluid containing the washings from the flies and the excreta passed by them was centrifuged. Five of the 36 collections (involving 1,362 flies) were found to contain *E. histolytica* cysts. In the second series of mass examinations, a total of 425 flies was collected indoors and divided into four lots each consisting of 80–125 flies. One of these lots was found to be positive, as judged by examination of the pooled intestines. The authors point out that *E. histolytica* cysts were obtained mainly from flies caught in houses where the inhabitants were known to be carriers, and whenever these cysts were found they were few in number and were possibly obtained from one or very few flies in each positive collection. Amongst recent writers who record successful infections of *M. domestica* with *E. histolytica* are Sieyro (1942) and Pipkin (1942). Pipkin, who recovered viable cysts, as judged by culture, from the faeces of *M. domestica*, also recovered them from the vomit-drop, as well as from the faeces, of *Chrysomyia macellaria*.

It can be seen from this survey of the literature concerning the rôle of the house-fly in the spread of amoebic dysentery that there exists a wealth of evidence derived from many sources that *M. domestica*, both in nature and in the laboratory, can take up the cysts of *E. histolytica* and subsequently excrete them in a viable condition in its faeces. It must not, however, be concluded from this that general agreement exists regarding the relative importance of the house-fly as compared with other methods of carriage of the cysts. Thus, Craig (1917), one of the early investigators on the association between the spread of amoebic dysentery and the prevalence of house-flies, is of the opinion that too much importance may be attached to the part played by house-flies in the spread of amoebic dysentery—a view also put forward by Woodcock in 1918—and that the main factors in the transmission of the disease are the pollution of water-supplies and the handling of food by persons who are symptomless carriers (Craig, 1935). It is also evident from the literature concerning bacillary and amoebic dysentery that, whereas the part played by both faeces and vomit-drop has been thoroughly investigated in the case of bacillary dysentery, the question of whether or not the vomit-drop plays a part in the transmission of amoebic dysentery has received but scanty attention.

#### MATERIALS AVAILABLE AND TECHNIQUE USED FOR EXAMINATION

Concentrated emulsions of *E. histolytica* cysts were prepared from the faeces of Service patients admitted to hospitals in Liverpool, the technique used being that described by Yorke and Adams (1926). Faeces from three different patients were used to prepare the concentrated emulsion, but in every instance these were subsequently diluted to a standard emulsion containing approximately three cysts in each 1/6 in. field. This concentration was checked before each experiment, and, although the distribution of the cysts varied somewhat, the concentration remained fairly constant in the different samples. The emulsions were stored in the refrigerator and used within 10 days of preparation, since it was found that up to that time there was no apparent change in the morphology of the cysts, though later there was a tendency for the cyst wall to shrivel, rendering it difficult to identify the cysts with certainty when searched for under experimental conditions. When feeding the flies, a drop of the emulsion was mixed with a minute trace of honey on a slide and offered to the fly. Since the concentration of cysts recorded from the excretions of flies is subject to many variable factors, no attempt was made to compare the exact number of cysts recovered with the numbers fed. A rough



estimate, however, can be made of the number of cysts which would have been present in the fly's excreta, had it fed under natural conditions, by dividing the numbers shown in the results by three, since the technique used usually resulted in a threefold concentration. All the flies used in the experiments were from a laboratory strain of *M. domestica* and were starved for 24 hours before being offered the *E. histolytica* emulsion. Two methods, illustrated in figs. 1 and 2, of feeding the flies were employed. In the first method, the fly was anaesthetized with ether, and the moment that it ceased to struggle the wings were cut off at the base, and the thorax of the fly was passed into a loop of 5 amp. fuse-wire carried in a capillary glass tube. The capillary was then fixed in a plasticine block in such a way that the fly was suspended in a natural resting position on a glass slide. When the fly had recovered from the anaesthetic—a process taking less than a minute—it was offered the *E. histolytica* emulsion. In the second method, each fly was placed in a separate rectangular glass box consisting of four glass microscope slides cemented together by their edges. One end was closed with gauze and the box was inverted over another slide containing a drop of the feeding-emulsion.

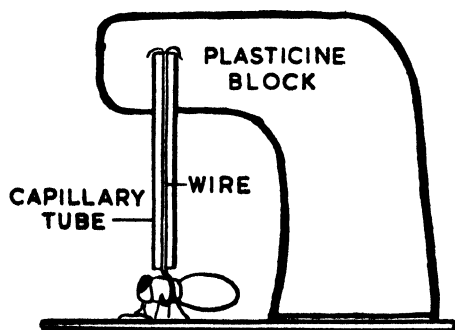


FIG. 1 Showing method of tethering flies employed in technique I.

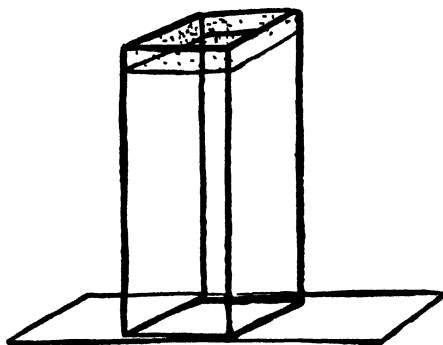


FIG. 2 Showing method of feeding used in technique II.

Both methods yielded equally satisfactory results so far as inducing the flies to take up the emulsion, but the latter or free method of feeding resulted in a much larger number of subsequent vomit and faecal excretions. Since the free method yielded more material, and since it imitated natural conditions more closely, it was the method most often employed. Whichever technique was used, the slide was removed after the fly had fed, and another slide, covered with a recently made blood film, was substituted. This simple technique proved very effective in inducing vomiting, presumably because the production of a vomit-drop was essential for the release of the red cells. In addition, it formed an admirable method of defining the feeding-site which later had to be searched for cysts, each of these sites being sharply limited by the punched-out area familiar to all who expose blood films in a fly-infested country. No special precautions were found necessary to mark the site of the faecal drops, which showed up clearly on the blood film. For the detection and enumeration of cysts in the excretions, a drop of Lugol's iodine was applied to the site, a cover-slip added, and the preparation systematically examined, using a high eyepiece and a 1/6 in. objective. When it was necessary to examine the gut contents, the fly was dissected in the usual manner, the appropriate part of the gut removed, and the

contents and scrapings examined in iodine. In most instances, no difficulty was experienced in detecting *E. histolytica* cysts and in distinguishing them from other cysts and from artefacts. When doubt arose, the object in question was omitted from the count.

When investigating the mechanism of feeding, as seen in the living fly when taking up and subsequently regurgitating food, an apparatus somewhat similar to that used by Lowne (1893-95) was employed. This consisted of a glass box constructed from slides cemented together to form a cube 1 in. by 1 in. by 1 in., which was fixed with Canada balsam on to another 3 in. by 1 in. slide, which formed the base. All these surfaces were painted black. The lid of the box was a cover-slip, 2 in. by 1 in., which was also blackened except for an area of 1 cm. by 1 cm. in the centre. The *E. histolytica* emulsion was placed on this clear area; a starved *M. domestica* was then introduced into the box, the lid was placed in position, and a beam of light was directed on to the box. The fly was then observed under the low-power lens of a binocular dissecting microscope.

### RESULTS OBTAINED

A survey of the literature, with which this paper begins, has shown that a number of competent investigators have studied, both in the field and in the laboratory, the carriage of *E. histolytica* cysts in the faeces of house-flies, and that the majority of these workers are in agreement that this vehicle plays an important part in the transmission of amoebic dysentery. On the other hand, the possible transmission of cysts by the vomit-drop has received scant attention, and the few authors who have recorded its examination have reported negative results. Under these circumstances, the following experiments were mainly designed to establish the rôle of the vomit-drop, but sufficient experiments were carried out in connection with the part played by the faeces to confirm the work of earlier writers.

#### I. THE PRESENCE, PERSISTENCE AND NUMBERS OF *E. histolytica* CYSTS FOUND IN THE FAECES OF HOUSE-FLIES PREVIOUSLY FED ON EMULSIONS OF THIS ORGANISM

(a) *Presence in the Faeces.* A total of 107 *M. domestica* were fed on the standard emulsion by one or other of the techniques described. Subsequent observations showed that the free flies yielded a larger number of faecal drops than did the tethered flies, and that all of the 68 flies which subsequently passed faeces were found to be excreting *E. histolytica* cysts in one or more of their droplets during an observation-period of 31 hours. The details of these results are recorded in Table I.

(b) *Persistence in the Faeces.* Various authors have noted that the excretion of cysts taken up at a single meal is distributed over a considerable period. Thus, Wenyon and O'Connor (1917) observed cysts in the faeces of flies from five minutes to 24 hours after feeding, while Root (1921) found that cysts persisted in the faeces for up to 49 hours. In our experiments, observations were not continued for more than 31 hours after the infective feed, during which time cysts were noted in the faeces as early as five minutes after they had been sucked up in the fly's proboscis, and were recorded at all times up to the end of the observation-period of 31 hours.

(c) *Numbers in the Faeces.* A total of 147 faecal drops containing 1,792 cysts were obtained from the 68 flies which passed faeces during an observation-period of 31 hours.

TABLE I

Showing the total number of flies fed on an *E. histolytica* emulsion, by two different techniques, and the proportion of the flies which subsequently passed faecal drops, together with the percentage of the total flies and total faecal drops from which *E. histolytica* cysts were recovered during an observation-period of 31 hours

Technique used	Total no. of flies fed	Total no and percentage of flies subsequently giving faecal drops	Total no of faecal drops passed	Average no. of faecal drops passed by each fly	No. and percentage of flies passing faecal drops containing cysts	Total no. and percentage of faecal drops containing cysts
I Fly tethered	87	48 (55%)	65	1.4	48 (100%)	65 (100%)
II Fly free	20	20 (100%)	82	4.1	20 (100%)	82 (100%)

The average number of cysts per faecal drop, therefore, was 12, or four if the fly had been feeding on the faeces before concentration. It is not possible to give figures for the maximum or minimum number of cysts in any one drop, since these were so often deposited in such close proximity as to render separate counts useless.

## II. THE PRESENCE, PERSISTENCE AND NUMBERS OF *E. histolytica* CYSTS FOUND IN THE VOMIT-DROPS OF HOUSE-FLIES PREVIOUSLY FED ON EMULSIONS OF THIS ORGANISM

(a) *Presence in the Vomit-Drops.* The same flies were used for the vomit-drop experiments as were employed in the faeces experiments, but the observation-period was limited to nine hours. The results obtained are shown in Table II.

It can be seen from the figures in Table II that 88 per cent. of the tethered flies and 100 per cent. of the free flies fed on the *E. histolytica* emulsion passed cysts in one or more of the vomit-drops deposited by them during the nine hours' observation subsequent to feeding. The figures also show that, as in the case of the faecal excretions, a greater number of vomit-drops was deposited by each of the free flies—an average of 15 vomit-

TABLE II

Showing the total number of flies fed on an *E. histolytica* emulsion, by two different techniques, and the proportion of the flies which subsequently passed vomit-drops, together with the percentage of the total flies and total vomit-drops from which *E. histolytica* cysts were recovered during an observation-period of nine hours

Technique used	Total no. of flies fed	Total no. and percentage of flies subsequently giving vomit-drops	Total no. of vomit-drops passed	Average no. of vomit-drops passed by each fly	No and percentage of flies passing vomit-drops containing cysts	Total no. and percentage of vomit-drops containing cysts
I Fly tethered	87	57 (88%)	72	1.3	49 (86%)	49 (70%)
II Fly free	20	20 (100%)	312	15	20 (100%)	150 (48%)

drops as compared with 1.3 from each of the tethered flies. It is of some interest to note that, although the tethered flies produced fewer vomit-drops, the proportion containing cysts was higher than among the free flies—70 per cent. as compared with 50 per cent. This difference could be due to the greater concentration of the cysts in the smaller number of vomit-drops passed, or to the lack of opportunity to clean the proboscis amongst the tethered flies as compared with the free flies—a point which will be further considered when the possible sources of the cysts are discussed.

(b) *Persistence in the Vomit-Drops.* In order to obtain records of the persistence of cysts in the vomit-drops of flies previously fed on an *E. histolytica* emulsion, observations were made over a period of nine hours after feeding. Unfortunately, the observation-period was not extended beyond nine hours, because at the time of these experiments the source of the cysts was believed to be the crop, and previous dissections had shown that the crop was always empty of its contents at the end of that time. As early experiments had shown that the free flies produced a larger number of vomit-drops than did the tethered flies, the former method was used to obtain the results which are recorded in Table III. This table shows the results of the examination of vomit-drops in the approximate order in which they were passed, but it must be emphasized that, owing to the rapidity with which they were deposited and to their close proximity, the precise order is probably subject to considerable error.

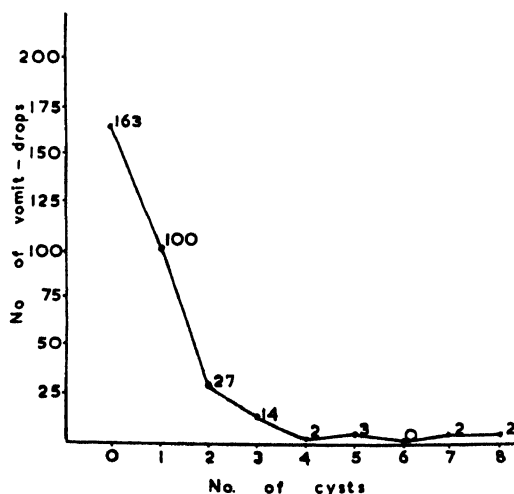
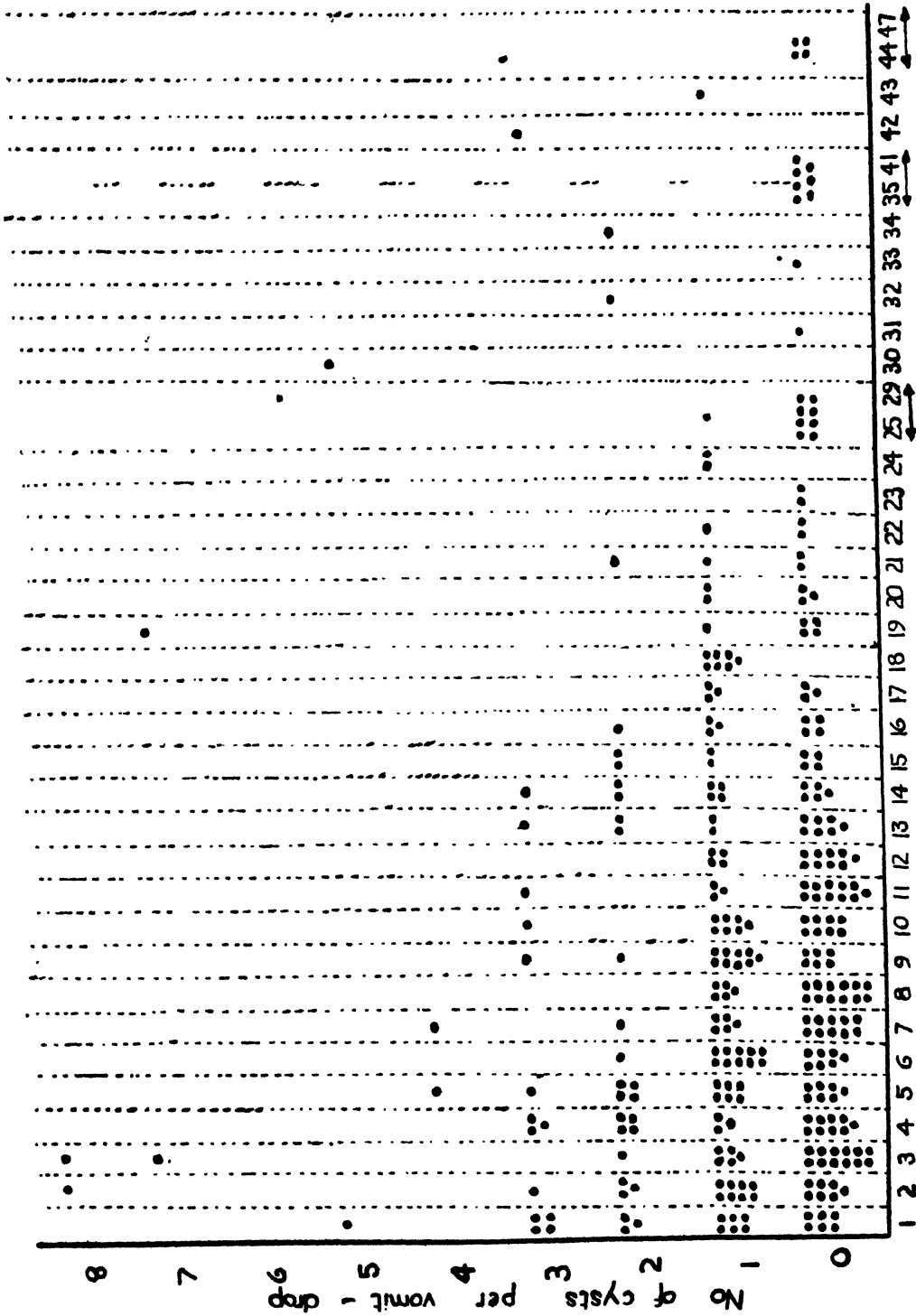


FIG. 3. Showing the frequency of vomit-drops containing 0-8 cysts.

It can be seen from Table III that *E. histolytica* cysts taken up by the house-flies appear at irregular intervals and in irregular numbers in the vomit-drops subsequently passed, but that there is a general tendency for the cysts to persist in the vomit-drops during the whole of the observation-period.

(c) *Numbers in the Vomit-Drops.* In the 312 vomit-drops obtained from 20 flies fed by technique II a total of 250 cysts was observed. The total number of vomit-drops and the total number of cysts obtained from each fly can be seen in Table III. Fig. 3 shows that 163 out of 312 (52 per cent.) of the vomit-drops obtained were negative for cysts,



### Approximate sequence of vomit - drops

Fig. 4. Showing the general trend in the numbers of cysts occurring in the first and subsequent vomit-drops obtained from a series of 20 flies. ● represents one vomit-drop.

TABLE III

Showing the number of *E. histolytica* cysts occurring in each approximately successive vomit-drop passed by 20 flies fed on an emulsion of the organism and then observed over a period of nine hours. The table also shows the total number of cysts deposited by the fly during the observation-period

Fly no.	No. of cysts obtained in vomit-drops in the approximate order in which they were deposited	Total no. of cysts from the fly	Total no. of vomit-drops from the fly	Total no. of positive vomit-drops from the fly
1	3 0 1 0 2 1 0 1 0 1 1 0 0 0 2 0 0 1 0 0 2	15	21	10
2	1 1 7 2 4 0 0 0 1 0 0 0	16	12	6
3	3 2 1 3 3 2 0 1 0 0 0 1 3 3	22	14	10
4	0 1 8 2 0 1 0 0 3 1 0 0 1 0	17	14	7
5	2 0 0 0 1 1 1 0 1 0 1 0 0 1 2 0	10	16	8
6	2 1 0 0 0 1 0 0 1 1	6	10	5
7	1 8 1 0 1 0 1 1 0 1 0 0 0 2 0 1 0 1 0 1 0 0 0 1 0	33	47	18
	0 0 0 0 5 0 2 0 2 0 0 0 0 0 0 0 3 1 0 0 0 0			
8	1 0 0 1 0 1 0 0 0 0 0 1 0 1 5 2 0 1 1 0	14	20	9
9	3 2 0 2 1 0 4 0 0 1 1 0 0 0	14	14	7
10	2 1 0 1 0 1 0 0 1 3 0 0 2 1 1 1 0 1 0	15	19	11
11	1 0 0 0 2 1 1 1 0 1 0 1 0 0 0	8	15	7
12	0 1 1 0 1 1 0 0 2 1 3 0 1 2 0 1 0 1 7 1 0 1 0 1 1	26	28	15
	0 0 0			
13	0 0 1 0 2 0 1 0 1	5	9	4
14	1 0 0 2 1 0 1 0 1	6	9	5
15	0 3 0 0 1 0 0 0 1 0 0 0 2 1 0 0 1 1 0 0 1 0	11	21	8
16	3 2 2 3 2 1 0 1 1 0 0	15	12	8
17	1 0 0 0 0 1 2 0 1 0 0 1 0 0 1 0 1	8	17	7
18	0 1 0 0 0	1	5	1
19	0 1 0	1	3	1
20	5 1 0 1 0 0	7	6	3
Totals		250	312	150

that 100 of the vomit-drops (35 per cent.) contained one cyst, 8 per cent. two cysts, and the remainder three to eight cysts. Whilst it was difficult in practice to keep an accurate record of the sequence in which the drops were discharged by each fly, it is possible, as is seen in fig. 4, to show approximately the general trend of the number of cysts occurring

in the first and subsequent vomit-drops. It is of particular interest to note that a high percentage of the first and subsequent vomit-drops obtained from a series of flies did not contain cysts, the figures available from the observations on the 1st-10th drops obtained from 16-20 flies varying from 30 to 70 per cent. (fig. 4).

### III. THE SOURCES FROM WHICH THE CYSTS ARE DERIVED

The results already recorded show that flies which have fed on an emulsion of *E. histolytica* cysts will subsequently deposit a proportion of these cysts in their vomit-drops. It appeared to be of interest to ascertain what proportion, if any, of these excreted cysts was derived from the dilatable part of the alimentary canal, i.e., the pharynx, oesophagus and crop, and what proportion was derived from the external surfaces and the internal channels of the proboscis. Until Graham-Smith (1930) described the method by which *Calliphora* sp. feed on material in varying physical states, it had been generally stated that the bulk of the food passed up the pseudotracheae, and that particles of diameter greater than that of the pseudotrachea ( $6\mu$ ) could only reach the gut by passing between the labellae and entering the alimentary canal directly along the main food-canal.\* That larger particles could enter the alimentary canal was demonstrated by Grassi (1883), who observed house-flies feeding on segments of *Taenia saginata* and on dissection found the eggs in the alimentary canal of the flies. Leon (see Galli-Valerio, 1910) fed flies on eggs of *Diphyllbothrium latum*, and later recovered them in the faeces of the flies, while Nicoll (1911) found that eggs of *Taenia solium*, *T. serrata*, *T. marginata*, *Hymenolepis nana*, *Dipylidium caninum*, *Diphyllbothrium latum*, *Enterobius vermicularis* and *Trichuris trichiura* could be recovered from the flies' intestines. This author concluded that only eggs less than  $50\mu$  in diameter could enter and pass through the alimentary canal of the fly. These observations were confirmed by Wenyon and O'Connor (1917), who examined the faeces of flies caught in the field and recovered eggs of *Trichuris trichiura*, *T. saginata* and *Ancylostoma* sp. and two eggs of bilharzia measuring  $130\mu$  by  $50\mu$ .

The experiments about to be described were undertaken in order to verify these earlier observations and to ascertain to what extent the nature of the food-emulsion alters the fly's method of feeding. The technique used for observing the proboscis of the fly during the act of feeding has been described in the introduction.

(a) *Method of Feeding on Liquid Food.* The liquid food consisted of the *E. histolytica* emulsion in the form of a small drop. After the fly had alighted on the slide, the proboscis was extended until it touched the surface of the drop. At this stage no opening between the labellae could be distinguished. Once feeding had commenced, a circular opening appeared between the labellae, which, although varying in its diameter, remained patent throughout the act of feeding. That fluid was being sucked up this opening with considerable force was demonstrated by the movement of the solid particles of the medium towards its centre, where the fluid could be seen to be drawn up in the form of a cone. While the labellae were still in contact with the food the proboscis was contracted and expanded rhythmically a few times, and was then lifted and replaced in another area of the emulsion. As the tip of the proboscis was removed from the food, a residue of debris which had remained attached to the end of the proboscis was dislodged and left on the slide

\* The term 'food-canal,' as used in this paper, denotes the channel formed by the hypopharynx and the labrum.

as a distinct particle, which, on subsequent examination, was found to contain a concentration of cysts. This description corresponds to the 'direct feeding' described by Graham-Smith (1930), but we made no observations regarding the action of the prestomal teeth, and it seemed to us that the labellar lobes, although not completely flattened, were always to some extent in contact with the emulsion during feeding.

(b) *Method of Feeding on Semi-Liquid Food.* The semi-liquid food consisted of a thick film of the emulsion which had been allowed to become viscid. The proboscis of the fly was extended as before, but the labellar lobes were much more extended, the size of the circular opening into the food-canal was reduced, and more food seemed to enter by way of the pseudotracheae than when the fly was feeding on the more liquid emulsion. Unfortunately, the presence or absence of cysts in the residue was not recorded. This account seems to correspond to Graham-Smith's 'intermediate position,' in which he described the method by which food passes directly into the food-canal as well as through the pseudotracheae. From these observations it seems that the difference between 'direct feeding' and 'intermediate feeding,' i.e., the methods by which the fly feeds on liquid and semi-liquid food, is only in the degree to which the labellae are flattened, the pseudotracheae are in contact with the food, and the food-canal is directly continuous with the food-material.

(c) *Method of Feeding on Dry Food.* The dry food consisted of a thick film which had been allowed to dry completely. The labellar lobes were completely extended, and a continuous excretion of liquid was maintained. The main food-canal was almost completely closed, and the food presumably passed up the pseudotracheae. As in the case of flies fed on liquid food, the residue was found to contain a concentration of cysts. This corresponds exactly with the 'filtering position' described by Graham-Smith.

The results of these experiments show that there are two main methods of feeding, which depend on the physical state of the food offered. It is not practicable to make a direct comparison with the observations of Graham-Smith, as his classification of media into semi-solid, tenacious, liquid, etc., is not easily comparable with our simpler classification into liquid, semi-liquid and dry food. Nevertheless, the results of these observations show clearly the method by which cysts of *E. histolytica* are taken up into the crop in such considerable numbers.

*Experiments to Determine (a) the Size of Organisms which, when Fed to the Fly, Subsequently Appear in the Crop, and (b) the Size of Organisms which, when Fed to the Fly, May Subsequently Appear in the Vomit-Drop*

In these experiments three emulsions were used: an emulsion of *E. histolytica* cysts (8–13 $\mu$ ), as employed in previous experiments, an emulsion of *E. coli* cysts (12–20 $\mu$ ), and an emulsion of oöcysts of *Eimeria acervulina* (22–25 $\mu$ ). Before the flies were fed, an estimate of the concentration of organisms in the emulsion was made by counting the number of cysts per field in a cover-slip preparation, when it was found that the *E. histolytica* emulsion contained three cysts per field, the *E. coli* emulsion eight cysts per field, and the *E. acervulina* emulsion 25 cysts per field. A total of 24 flies were fed, by means of the free-fly method, eight flies being fed on each organism. Five flies in each group were dissected immediately after feeding, while the remaining nine flies (comprising three flies in each group) were kept alive and the vomit-drops which they deposited were examined for the presence of cysts.



(a) *The Size of Organisms which, when Fed to the Fly, Subsequently Appear in the Crop.* The crops of the five flies fed on an emulsion of *E. histolytica* cysts, and dissected immediately after feeding, were all found to contain cysts in an average concentration of four cysts per field, as compared with three cysts per field in the original emulsion; it is possible that this small increase was due to absorption of fluid from the crop. The crops of the five flies fed on the *E. coli* emulsion also contained cysts, but in the case of these larger cysts the concentration was reduced to six per field, as compared with eight per field in the original. In the case of the five flies fed on *E. acervulina*, oöcysts were present in the crop of each fly but in greatly reduced numbers—10 per field, as compared with 25 per field in the original emulsion. These results suggest that during the passage of food to the crop there is a filtering off of particles greater than  $12\mu$ , only 40 per cent. of organisms 22–25 $\mu$  in diameter reaching the gut.

(b) *The Size of Organisms which, when Fed to the Fly, May Subsequently Appear in the Vomit-Drop.* In previous experiments it has been shown that, when flies are fed on *E. histolytica* emulsions, some debris adheres to the proboscis, and it might therefore be thought that some or all of the cysts found in the experiments described above were derived from this source. This is unlikely, however, since it has been observed that, after a meal and before vomiting has commenced, the fly always cleans the proboscis with sufficient care to remove at any rate most of the debris. The three flies which fed on *E. histolytica* cysts and which were subsequently observed passed a total of 57 vomit-drops, containing 30 cysts. In contrast to this, only one *E. coli* cyst ( $12\mu$  in diameter) was found in the total of 51 vomit-drops passed by the three flies fed on an emulsion of this organism, while no oöcysts were found in any of the 47 vomit-drops deposited by the three flies fed on an emulsion of *E. acervulina*. These results suggest that the majority of the *E. histolytica* cysts which enter the crop are held back by the pseudotracheae when the fly vomits.

The source of the cysts which do appear in the vomit-drop is doubtful; they may, on the one hand, be derived from the crop and be regurgitated down the food-canal and to the exterior through the opening to this canal between the labellae; but it is more probable that they are not derived from the crop at all but are cysts which have become lodged in the pseudotracheae and remain there until flushed out by the fluid from the crop or the salivary glands. This, of course, is only a conjecture, but, if the *E. histolytica* cysts seen in the vomit-drop had descended from the crop through the main food-canal, there seems no obvious reason why a proportion of the larger species of cysts could not have followed a similar route, nor why so many of the early vomit-drops of a series from a fly should be negative for cysts of *E. histolytica*. On the other hand, if we accept the view that the *E. histolytica* cysts which appear in the vomit-drop are those which became wedged in the pseudotracheal openings until washed out by fluid from the oesophageal diverticulum, then the virtual absence of *E. coli* and *E. acervulina* could be explained by the argument that they were too large to enter the pseudotracheal openings.

## DISCUSSION

Both the survey of the literature and the results of the experiments which we have carried out show that a fly, given access to infected material, may be responsible for the transmission of *E. histolytica* cysts in its faeces. Our experiments have shown that the period during which the fly can be regarded as a source of infection by this route may extend from five minutes to at least 31 hours after the initial infective feed—the limit

of our observations—and that during this period cysts are excreted in considerable, though irregular, numbers. In the main, the observations recorded in this paper confirm those of earlier writers regarding the route taken by the ingested cysts which subsequently appear in the faeces, and it would seem that most, possibly all, of the *E. histolytica* cysts which enter the crop pass up the main food-canal, subsequent passage through the gut being completed by individual cysts at widely varying intervals.

The experiments described in the present paper have been concerned mainly with the part played by the vomit-drop in the spread of *E. histolytica* cysts, a subject which has, up to the present time, received very little attention. The results of these experiments prove that *E. histolytica* cysts taken up by house-flies during the act of feeding subsequently appear in their vomit-drops, at irregular intervals and in irregular numbers, throughout an observation-period of nine hours after the initial infective feed. The route taken by the cysts occurring in the vomit-drop has not been ascertained with certainty. As a result, however, of observations made on the fly during the act of feeding and on the effect on the size of the organisms fed to the fly in determining their subsequent appearance in the vomit-drop, it is possible to put forward the following provisional hypothesis. As the distal opening of the food-canal is closed during vomiting, it would appear that few, if any, of these cysts are derived from the crop, and that, whereas cysts of  $12\mu$  and greater, such as *E. coli* and *E. acervulina*, cannot enter the aperture of the pseudotracheae, the smaller *E. histolytica* cysts can do so, becoming wedged in the pseudotracheal channels and remaining there until washed out by fluid from the crop or from the salivary glands. It is possible, however, that a few of the cysts which are regurgitated with the crop contents enter the pseudotracheae from above, and are gradually washed down the pseudotracheae with successive discharges of fluid from the crop.

It is now generally believed that, in urban areas, polluted drinking-water and food-handlers are the main sources of infection with amoebic dysentery (Craig, 1935; United States Public Health Service, 1936). In rural areas, however, particularly in the tropics, flies may prove of greater importance, for, under such conditions, not only do they tend to occur in greater numbers, but owing to lack of sanitation they have readier access to infected excreta and to human food-supplies. It is difficult to estimate the relative importance of the faecal drops and the vomit-drops as sources of infection, for, whereas the faeces undoubtedly contain a larger total of cysts, they are produced less frequently.

The results recorded in the present paper not only confirm the work of previous writers regarding the number and persistence of *E. histolytica* cysts in the faeces of flies having access to stools containing these organisms, but show that the cysts are also deposited in the vomit-drops; the number of cysts dispersed by flies and consequently the risk of contamination of fly-frequented surfaces are greater than was previously believed.

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# AN INVESTIGATION INTO THE EFFECTS OF 'GAMMEXANE' ON THE LARVAE, PUPAE AND ADULTS OF *CULICOIDES IMPUNCTATUS* GOETGHEBUER AND ON THE ADULTS OF *CULICOIDES OBSOLETUS* MEIGEN

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## INTRODUCTION

Extensive field studies upon the life-histories of several British species of *Culicoides* were carried out by one of us (M. A. H.) in 1945 and 1946, and a full account of these observations is given by Hill (1947). The present paper is concerned with experiments made during the summer of 1946 to investigate the possible uses of 'Gammexane' ‡ for the control of *Culicoides*.

A survey of the literature on the control of *Culicoides* shows that remarkably little information is available. As regards methods to prevent breeding, the only conclusive experimental work is that which has been done against species inhabiting the salt-marshes of Florida, where considerable success has been achieved by draining the marshes. The most recent report, by Hull, Shields and Platts (1943), records a reduction of 90.1 per cent. in the period July, 1939, to September, 1940, and a reduction of 72.9 per cent. in the period November, 1940, to November, 1941, in the numbers of larvae isolated from drained marshes as compared with the numbers isolated from undrained marshes. In addition, the number of adult midges which emerged from the drained marshes was 90.65 per cent. lower in 1939-40, and 82.78 per cent. lower in 1940-41, than the numbers emerging from undrained marshes. Drainage, combined with flooding and the filling in of shallow lagoons, is advocated by Painter (1926) for the control of *Culicoides* in the Bahamas. With regard to chemical control, Dove, Hall and Hull (1932) report phenolized pine-sap as a promising larvicide for the control of breeding in *Culicoides*. In recent years much attention has been paid to the insecticidal properties of DDT and 'Gammexane,' but we are aware of only one series of experiments (Steward, 1946a, 1946b) on the effects of DDT and 'Gammexane' on *Culicoides*. Working with larvae only, Steward used a miscible-oil preparation (PD/RS/474/45) containing 'Gammexane' and a similar preparation of DDT (PD/RS/763/45), and tested the effect of these preparations at various concentrations on the larvae of *C. nubeculosus* in the laboratory. The number of larvae used was small, but he reports that three out of three larvae were killed after immersion for 24 hours in DDT at a concentration of 1/10,000, though a concentration of

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‡ In this paper the word 'Gammexane' means the gamma isomer of benzene hexachloride.

1/5,000,000 had little effect over the same period. After similar tests with 'Gammexane,' it appeared to him that this compound was more effective than DDT against the larvae of *C. nubeculosus*. Thus, 'Gammexane' at concentrations of 1/10,000 and 1/5,000,000 killed three out of three larvae after they had been immersed for 24 hours, and even at 1/8,000,000 three out of three larvae became moribund. The control of breeding in *Culicoides* clearly offered scope for further study, and accordingly the experiments reported below were undertaken.

## THE EXPERIMENTS

The experiments consisted of two main parts. The more intensive study was made over a period of four months on the effects of 'Gammexane' on the larvae and pupae of *Culicoides*, and towards the end of the season a number of observations were made on the effects of the insecticide on the adult midges. The design of the experiments, the methods employed, and the results obtained for the immature stages and for the adult midges are discussed separately.

### I. THE INVESTIGATION INTO THE IMMEDIATE AND RESIDUAL ACTION OF 'GAMMEXANE' ON THE LARVAE AND PUPAE OF *Culicoides*

*The General Scope of the Experiment and the Choice of Species.* It was decided that the experiment on the control of breeding in *Culicoides* by 'Gammexane' should include observations under field conditions combined with supplementary laboratory tests. *Culicoides impunctatus* was selected as the most suitable species for the work, as adequate breeding-places were known and the life-history and habits of the species—particularly the seasonal fluctuations in the density of the larvae and pupae—had been studied for a year under normal conditions by the senior author (Hill, 1947).

*Choice of Site.* In the 1945 survey (Hill, 1947), the larvae and pupae of *C. impunctatus* were found in wet, but not necessarily waterlogged, soil rich in humus under the shade of rhododendron bushes at White Man's Dam in Knowsley Park, Liverpool. The bushes provided a leafy canopy, approximately 6 in. above ground-level. Six similar samples of soil were taken from the area and examined for *Culicoides* larvae and pupae during each fortnightly period from March, 1945, to March, 1946. In this way a record was obtained of the changes in the density of the larvae and pupae of *C. impunctatus* in one breeding-ground throughout a complete year. Provided that the site chosen for the experimental work on the effect of 'Gammexane' on the immature stages of *C. impunctatus* was of a similar nature, it was considered that this record for normal conditions would be invaluable in assessing the effects of experimental treatment. A site similar to that near White Man's Dam was ultimately selected under rhododendron bushes along the shore of Mizzy Dam, Knowsley Park, Liverpool. In this area three plots, numbered 1, 2 and 3, approximately 20 ft. long by 4 ft. wide and separated by a 20 ft. gap, were marked out with string and metal skewers. These were selected so that the shade of the over-hanging rhododendron bushes extended along the whole length of each plot and over about half the width. It was observed that in each plot the larvae and pupae were present only in the shaded areas, and that no immature stages were to be found in a strip about 9 in. in width furthest from the shade. For the purpose of the experiment this was a convenient distribution of the larvae and pupae.

*Time of the Experiment.* During 1945 it was found that there was one generation of adults of *C. impunctatus* in a year, and that the adults began to emerge about the beginning of May. It seemed probable, therefore, that treatment with 'Gammexane' might be most effective if carried out at the time when the larvae were pupating. Thus it was supposed that not only would the larvae and pupae present at the time of the treatment be exposed to the immediate insecticidal action of the 'Gammexane,' but also that, even with only one application, the residual action might later prove effective against ovipositing females, their eggs or the young larvae derived from the eggs. In 1946, sampling showed that the majority of the larvae were beginning to pupate during the third week in May—rather later than in 1945—and therefore spraying of the experimental plots was carried out on May 21st, before many adults had emerged. From then onwards, as described below, both field and laboratory observations were made until September 24th, a period of four months covering the active breeding-season of *C. impunctatus*.

*The Concentration and Formulation of the 'Gammexane' Deposit and of the Control Deposits, and the Method of their Application to the Experimental Plots.* At the time when the experiment was planned we had not traced any literature concerning the application to the soil of a solution containing 'Gammexane,' and we therefore had little information to guide us in selecting a suitable concentration of the insecticide. Thomas and Jameson (1946) applied the insecticide to the soil in a dust diluent at concentrations of less than 10 mgm. 'Gammexane' per sq. ft., and, although they report a 65 per cent. decrease of wireworms, no particulars are given of any residual action. Mitchell (1946) found that 'Gammexane' in china-clay, distributed, at concentrations up to 10.4 mgm. per sq. ft., against tobacco-grubs in the soil, produced a reduction, in 6–7 weeks, in the number of grubs as compared with untreated areas. This result was mainly apparent at the highest concentration of 10.4 mgm. per sq. ft. Golightly (1946) also used a preparation of 'Gammexane' against the wireworm, and found that 2 cwt. per acre, broadcast, produced 100 per cent. mortality of wireworms, and that 100 per cent. of oats sown in soil treated in this way grew to maturity; but he does not state the percentage of 'Gammexane' in the material with which he treated the soil. Dawson and Escritt (1946) tested 'Gammexane' against the leather-jacket, in the form of powders containing 3½ per cent. and 5 per cent. benzene hexachloride, containing 13 per cent. gamma isomer, and found that complete control was obtained after the application of 1 oz. of the 3½ per cent. formulation per sq. yd., thus giving a concentration of 0.0045 oz. or 14 mgm. 'Gammexane' per sq. ft. Since we hoped to achieve an immediate control of *Culicoides*, combined with a residual action, it was decided to apply 'Gammexane' in our experiment on one occasion only and at a concentration of 100 mgm. per sq. ft. of breeding-ground.

A solution of 5 per cent. 'Gammexane' in miscible oil (MG 045) was used for the experiment, preliminary laboratory tests having shown that the miscible-oil base had no apparent toxic effect on the larvae of *C. impunctatus*. To obtain the required deposit of 100 mgm. 'Gammexane' per sq. ft. of breeding-ground, it was necessary to distribute 160 c.cm. of the 'Gammexane' solution (MG 045) over the 80 sq. ft. area of a plot. It was found by preliminary trial that, with the use of a 'Mysto' knapsack sprayer, at an initial pressure of 65 lb. per sq. in., 160 c.cm. of the 'Gammexane' solution (MG 045), made up to 4 litres with water, was a convenient quantity of liquid to spray over the specified area. The three plots at Mizzy Dam, each of 80 sq. ft. surface area, and designated plots 1, 2 and 3, were sprayed as follows :

Plot 1 (control 1): 4 litres of lake-water.

Plot 2 (control 2): 160 c.cm. of the miscible oil, plus 3,840 c.cm. of lake-water.

Plot 3: 160 c.cm. of 5 per cent. 'Gammexane' in the miscible oil (MG 045), plus 3,840 c.cm. of lake-water ( $\equiv$  100 mgm. 'Gammexane' per sq. ft.).

*The Methods of Sampling and the Sequence of Observations on the Effects of the 'Gammexane' Treatment and the Control Treatments.* The observations made on the effects of the different treatments on the larvae and pupae of *C. impunctatus* are considered under three headings:

1. Field tests.
2. Supplementary laboratory tests.
3. Soil-penetration tests.

The methods used in each type of test are dealt with below.

1. *Field Tests.* A field test consisted of taking one or more samples of soil from a plot and examining it to observe the numbers and vitality of the larvae and pupae present. Each soil-sample consisted of a sod, 6 in. square and 1 in. deep, removed from the soil in the shaded area of the plot where immature stages were known to occur. The survey made in 1945 had shown that 90 per cent. of the immature stages occurred in the surface inch of the soil. The method used for the isolation of the immature stages from the soil has been described by Hill (1947). By this method two samples were taken from each of the three plots on the day preceding treatment and 1-3 samples approximately daily up to the 13th day following the treatment on May 21st. Thereafter, one sample was examined from each plot on the 14th, 20th and 22nd day after treatment and, except during July, at approximately weekly intervals until the 126th day, namely, September 24th.

In view of the information available from the 1945 survey (Hill, 1947) concerning the normal seasonal fluctuations in the density of the immature stages of *C. impunctatus*, and since the experimental plots treated with lake-water only and miscible oil only were both sources of data for control observations, it was considered advisable to take more samples from the 'Gammexane' plot than from either of the other two plots during the first fortnight of the experiment. Details, for each plot, of the number of samples per observation and of the counts for each sample are given in Tables I, II and III. Graphs 1-3, showing the results for the plots at intervals throughout the experiment, have been constructed by taking either the total count for one sample or the average number of immature stages present in two or three samples, as the case may be. In general, it was found that the graphs were more simply constructed by combining the numbers of larvae and pupae, dead or alive, in a sample as 'total immature stages.'

2. *Supplementary Laboratory Tests.* In this series of tests it was desired to take samples of soil from each of the treated plots and to test the samples for their toxicity to 10 introduced healthy larvae of *C. impunctatus*. Samples of top soil from each plot were taken at intervals throughout the four months of the experiment from the unshaded strip where no larvae had been found. Each such sample was divided into blocks of soil, 5 cm. by 5 cm. by 1 cm. thick, and these were teased out in the laboratory into a Petri dish containing 40 c.cm. of tap-water. Ten third or fourth stage larvae of *C. impunctatus* were added to each Petri dish. The number of days required to give a 100 per cent. kill of larvae in the dish of soil from the 'Gammexane' plot was recorded and compared with the vitality, after the same interval, of the larvae exposed to contact in a similar way with soil from the control plots. On the occasion of each weekly test enough blocks were

set up in this way from each plot to enable a daily examination to be made of one block per plot until a kill of 100 per cent. larvae in the 'Gammexane' soil-sample was recorded.

3. *Soil-Penetration Tests.* A few tests were made to estimate the depth of soil to which the 'Gammexane' penetrated in sufficient concentration to be toxic to third or fourth stage larvae of *C. impunctatus*. For this purpose, samples of soil from the plot treated with 'Gammexane' were taken at various depths and the toxicity was tested by introducing 10 healthy *C. impunctatus* larvae into Petri dishes containing water and teased-out blocks of the soil, as described for the supplementary laboratory tests.

TABLE I

Showing that on the day before the experimental treatment plots 1, 2 and 3 contained only healthy larvae and pupae of *C. impunctatus* and that these were fairly evenly distributed in the three plots

Plot no.	Sample	No. of larvae			No. of pupae			Total immature stages		
		Alive	Dead	Average per sample	Alive	Dead	Average per sample	Alive	Dead	Average per sample
1	1	10	0	11	3	0	3	13	0	14
	2	12	0		3	0		15	0	
	Totals	22	0		6	0		28	0	
2	1	9	0	10	4	0	3	13	0	13
	2	11	0		2	0		13	0	
	Totals	20	0		6	0		26	0	
3	1	14	0	12	3	0	4	17	0	16
	2	10	0		5	0		15	0	
	Totals	24	0		8	0		32	0	

## RESULTS

*The Density of the Immature Stages of C. impunctatus in Plots 1, 2 and 3 Before Treatment*

The results of sampling, by the method described above under the heading 'Field Tests,' for the immature stages of *C. impunctatus* in the shaded part of the three plots before the experimental treatment was applied are shown in graphs 1-3 and Table I. The results indicate a similar proportion of larvae and pupae in each of the three plots, with an average of 14, 13 and 16 total immature stages per sample from plots 1, 2 and 3 respectively.

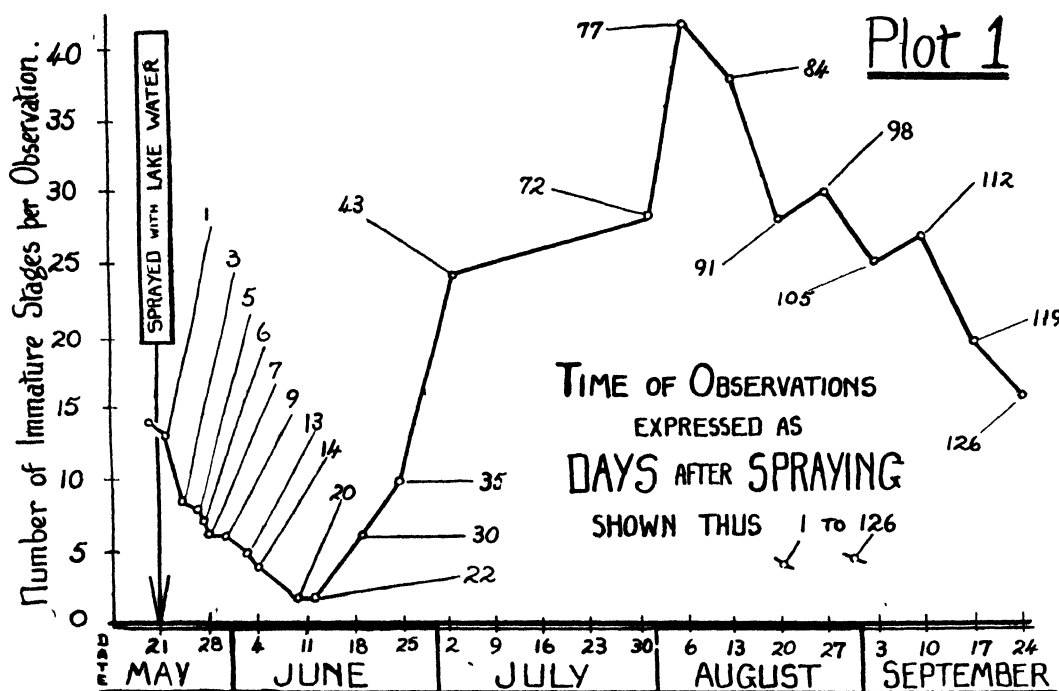
*The Immediate and Residual Effects of 'Gammexane' at a Deposit of 100 Mgm. per Sq. Ft. on the Larvae and Pupae of C. impunctatus*

*Field Tests.* The results of sampling to observe the numbers and vitality of the larvae and pupae of *C. impunctatus* in the three experimental plots at various intervals after treatment on May 21st are shown in graphs 1-3 and in Tables II-IV (from which data the graphs were constructed).

It will be observed that, up to the 20th day after spraying, there was, in all three plots, a fall in the total number of immature stages recovered per sample. The 'Gam-



mexane'-treated plot was negative for larvae and pupae by the 13th day and remained so during the four months of observations. Both the control plots, however, while maintaining a rather low density of immature stages for a further 15 days, exhibited thereafter a marked increase in the numbers of larvae. This increase reached a recorded maximum on the 77th day after spraying, and by the 126th day had again fallen slowly to a larval count per sample approaching that recorded at the time of spraying. Pupae ceased to occur in the control plots about the 43rd day of these observations. As regards vitality, it is of particular interest to note that throughout the experiment only live larvae and pupae were taken in the control plots. On the other hand, as is seen in Table II, in the first five days after treatment only 23.8-55.6 per cent. of the larvae and 61.5-90.9 per cent. of the pupae taken from the 'Gammexane' plot were alive, and, following a



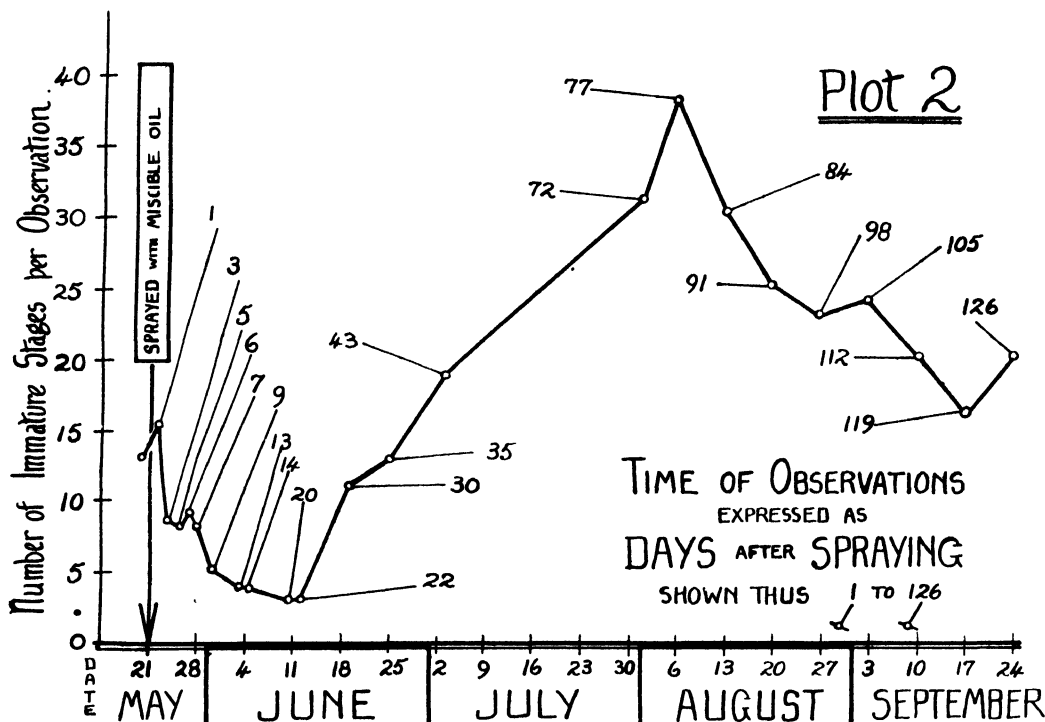
GRAPH 1. Showing that the density of the larvae of *C. impunctatus* in breeding-grounds during the active breeding-season for the species was not affected by a single application in May of the natural lake-water used in diluting the 'Gammexane' preparation in the experiment.

heavy downpour of rain on the evening of the fifth day, only dead larvae or dead pupae were recovered from this plot. Of a total of 67 pupae collected from the 'Gammexane' soil in the first nine days of the experiment, none produced adults, whereas there was a 100 per cent. emergence of adults from 65 pupae collected in the same period from a comparable number of samples from the combined control plots (Table IV).

The number of larvae taken per sample is not large, but it will be noted that the percentage of live larvae from the 'Gammexane' plot on the fifth day (55.6 per cent.) is larger than in the three earlier observations (23.8-38.5 per cent.). It will also be

observed that the percentage of live pupae (61.5-90.9 per cent.) was greater for these samples than the corresponding percentage of live larvae (23.8-55.6 per cent.).

**Supplementary Laboratory Tests.** The observations made in the laboratory to confirm the field results on the toxicity of the 'Gammexane' to the immature stages of *C. impunctatus* were carried out at intervals throughout the four months' period of the experiment. In every test the dishes containing the soil from the control plots showed that at least 8 out of 10 introduced *C. impunctatus* larvae were normal after the same time-interval required to effect a 100 per cent. kill of the same number of larvae introduced to corresponding dishes containing soil from the 'Gammexane'-treated plot. The results obtained by introducing larvae to the samples of soil treated with 'Gam-



GRAPH 2. Showing that the density of the larvae of *C. impunctatus* in breeding-grounds during the active breeding-season for the species was not affected by a single application in May of the miscible-oil base of the 'Gammexane' preparation used in the experiment.

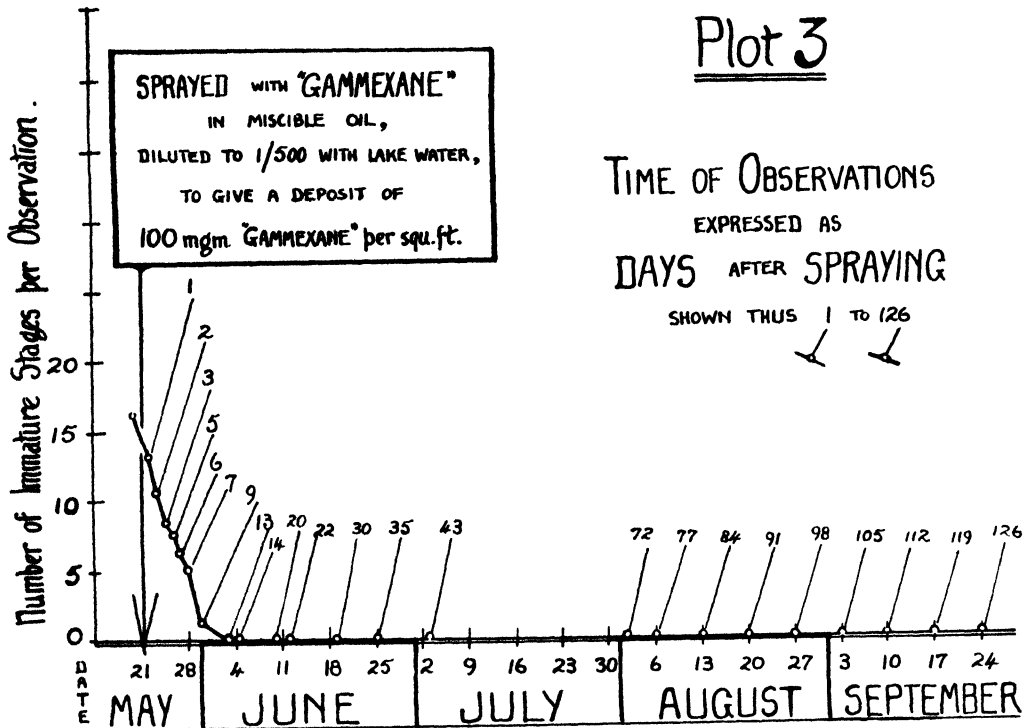
mexane' are shown in graph 5. This shows that, for the first two weeks after the insecticide had been applied, a period of 24 hours elapsed between the introduction of the larvae and their death. However, immersion for 48 hours was necessary to obtain a 100 per cent. kill of the larvae introduced to soil-samples taken three and four weeks after treatment. A continued increase in the time required to obtain a 100 per cent. kill of larvae is apparent up to the last observation in the 17th week, when seven days' immersion was necessary to give a complete kill.

**Soil-Penetration Tests.** In connection with the deposit on plot 3 of 100 mgm. 'Gammexane' per sq. ft. of ground, a number of tests were made to assess the depth





to which the 'Gammexane' penetrated the soil in a concentration sufficient to be toxic to *C. impunctatus* larvae. The technique used was the same as that employed in the supplementary laboratory tests. It was found that, for the first five days after spraying, the soil was toxic to larvae for a depth of only 1 cm. below the surface. After the heavy rain at the end of the fifth day, and throughout the summer, when the soil became progressively waterlogged (graph 4), 'Gammexane' was found to be present to a depth of 3 cm. below the surface in a concentration toxic to *C. impunctatus* larvae. This condition prevailed up to the last test on the 126th day of the experiment (September 24th).



GRAPH 3. Showing that 'Gammexane' applied once in May at a concentration of 100 mgm per sq. ft of breeding ground prevents the development of immature stages in the treated soil during the remainder of the active breeding-season of *C. impunctatus* up to September 24th.

#### DISCUSSION

As will be seen from graphs 1-3, the progressive decrease, during the three weeks following treatment on May 21st, in the numbers of immature stages in each of the control plots follows closely a similar reduction in the number of immature stages recorded from the 'Gammexane' plot. Such reduction in the total numbers of larvae and pupae as each of the three treatments may have caused is, however, obscured by a natural fall in the density of larvae and pupae at this time of the year. It has been shown by Hill (1947) that in May particularly the over-wintering larvae of *C. impunctatus* are pupating, and that in June the adults are emerging from the pupae in large numbers, with a consequent reduction for that month in the number of late larval stages and pupae present in the soil.

TABLE III

Showing that, after the treatment of plot 1 and plot 2 on May 21st, no dead immature stages of *C. impunctatus* occurred, and that the density of larvae and pupae was similar in both plots and showed seasonal fluctuations characteristic of normal conditions. All pupae taken from plots 1 and 2 produced adults

Date	Day after treatment	Plot 1, sprayed with lake-water only						Plot 2, sprayed with miscible oil only					
		No. of samples	No. of live larvae*	Pupae		Total immature stages		No. of samples	No. of live larvae*	Pupae		Total immature stages	
				No.* alive	Percent. adults emerged	No.* alive	Average per sample			No.* alive	Percent. adults emerged	No.* alive	Average per sample
22.5.46	1st	2	7 8	5 6	100	12 14	13	3	8 9 6	6 4 3	100	14 13 9	12†
		Totals	15	11		26		Totals	23	13		36	
23.5.46	2nd	No observation						No observation					
24.5.46	3rd	2	5 4	3 5	100	8 9	8.5	2	8 3	4 2	100	12 5	8.5
		Totals	9	8		17		Totals	11	6		17	
26.5.46	5th	1	5	3	100	Total per single sample			8 2	4 2	100	12 4	8
								Totals	10	6		16	
Heavy rain													
27.5.46	6th	1	4	3	100	7		1	5	4	100	Total per single sample	
28.5.46	7th	1	3	3	100	6		1	4	4	100	9	
30.5.46	9th	1	4	2	100	6		1	3	2	100	8	
3.6.46	13th	1	3	2	100	5		1	2	2	100	5	
4.6.46	14th	1	2	2	100	4		1	3	1	100	4	
10.6.46	20th	1	1	1	100	2		1	2	1	100	4	
12.6.46	22nd	1	1	1	100	2		1	1	2	100	3	
19.6.46	30th	1	5	1	100	6		1	10	1	100	3	
25.6.46	35th	1	10	0	—	10		1	13	0	—	11	
3.7.46	43rd	1	23	1	100	24		1	18	1	100	13	
1.8.46	72nd	1	28	0	—	28		1	31	0	—	19	
6.8.46	77th	1	42	0	—	42		1	38	0	—	31	
13.8.46	84th	1	38	0	—	38		1	30	0	—	38	
20.8.46	91st	1	28	0	—	28		1	25	0	—	30	
27.8.46	98th	1	30	0	—	30		1	23	0	—	25	
3.9.46	105th	1	25	0	—	25		1	24	0	—	23	
10.9.46	112th	1	27	0	—	27		1	20	0	—	24	
17.9.46	119th	1	20	0	—	20		1	16	0	—	20	
24.9.46	126th	1	16	0	—	16		1	20	0	—	16	

\* No dead larvae or pupae found in samples from plots 1 and 2.

† This record is shown incorrectly as 15.3 in graph 2.

There is, however, as shown in Table II, a considerable death-rate during the first nine days of the experiment amongst the larvae and pupae exposed to contact with 'Gammexane,' whereas in the control plots (Table III) only living larvae and pupae occur. Furthermore, as is shown clearly in Table IV, no adults emerged from the live pupae collected from the 'Gammexane'-treated soil, although there was a 100 per cent. emergence of adults from the pupae found during the same period in the two control plots. Thus, despite the occurrence of a natural decline in the numbers of larvae and pupae present in the breeding-grounds during that time of the year, there is convincing evidence from a consideration of the vitality of the immature stages that the 'Gammexane' exerted an effective insecticidal action on both the larvae and the pupae of *C. impunctatus* for the first nine days after the application of the insecticide.

The percentage of live larvae recorded from the 'Gammexane' plot during the first five days varied from 27.3 per cent. on the first day after spraying to 55.6 per cent. on the

TABLE IV

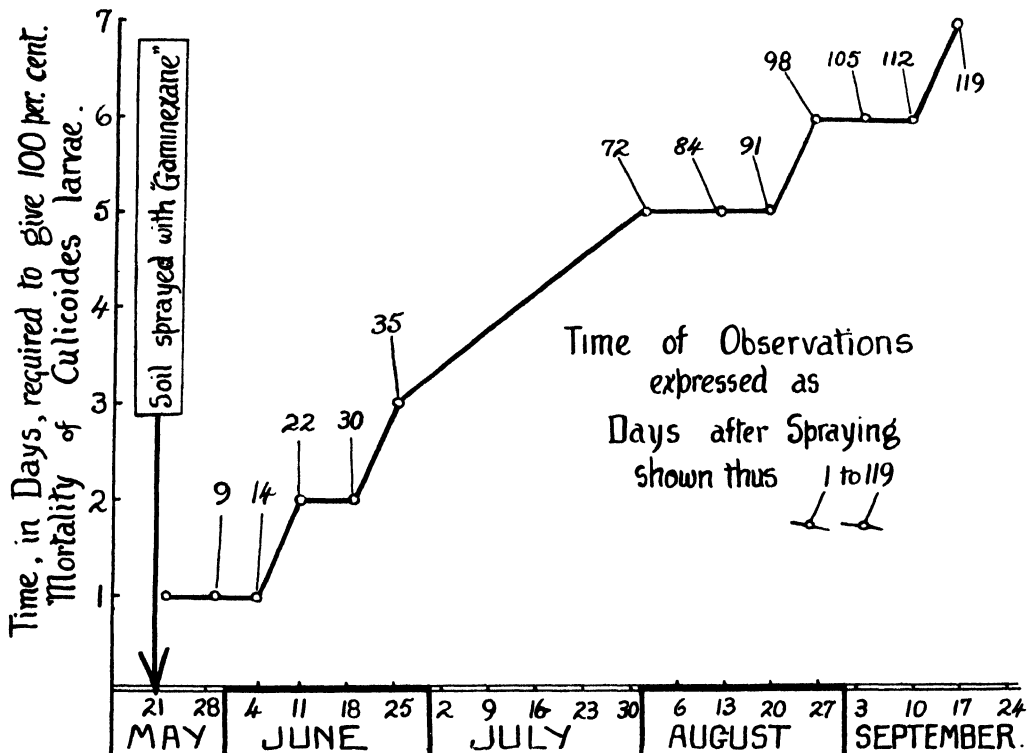
Showing the total number of pupae of *C. impunctatus* taken in the samples from plots 1, 2 and 3 from the 1st day to the 9th day after treatment, and that only in the 'Gammexane'-treated plot was there any toxic action on the pupae as expressed by the number of dead pupae taken at sampling and the percentage of adults emerging from live pupae taken at sampling

Plot	Total no. of samples taken containing pupae	Total pupae	Total live pupae at sampling	Percent. of dead pupae in total pupae taken at sampling	Percent. of adults later emerging from pupae alive at sampling
1 (lake-water only)	8	30	30	Nil	100
2 (miscible oil only)	10	35	35	Nil	100
1 and 2 combined	18	65	65	Nil	100
3 ( 'Gammexane' in miscible oil at 100 mgm. 'Gammexane' per sq. ft.)	19	67	43	35.8	Nil

fifth day. Though the number of larvae per sample is admittedly small, the figures suggest that the 'Gammexane' was becoming, in a short time, progressively less effective against the larvae. In view of the fact, however, that we could not identify larvae which had been dead in soil for more than 48 hours, this apparent increase in the percentage of surviving larvae is almost certainly spurious, and does not allow for larvae which had died but were unrecognizable. Nevertheless, it is clear that the immediate action of the 'Gammexane' was not complete and that larvae survived up to five days after treatment. How long the pupae survived must remain uncertain, as the date of pupation for any one pupa is not known.

From the recovery of higher percentages of live pupae than live larvae in the first five days of the experiment it may seem that the pupae are more resistant to 'Gammexane.' Pupae occur on more elevated particles of the soil, whereas larvae tend to be in the hollows. It may be argued that the pupae would be exposed to a more direct and intimate contact with the insecticide at the time of spraying, and, if this is so, then it

would be true that the pupae are more resistant than the larvae. On the other hand, it is not improbable that a greater volume of the spray would collect in the lower hollows, resulting in a relatively higher deposit of 'Gammexane' there than on the higher particles. By this view, the greater percentage of live pupae would be due, not to their resistance, but to the fact that they had been exposed to a relatively lower concentration of 'Gammexane' than the larvae. It seems to us inappropriate to discuss further the relative resistance of larvae and pupae, as the data available from these experiments are concerned, in general terms only, with the distribution on the surface and with the depth of penetration of the 'Gammexane.'



GRAPH 4. Showing that soil treated with 'Gammexane' at 100 mgm. per sq. ft. retains, under the field conditions of the experiment, a diminishing but effective residual action against third and fourth stage larvae of *C. impunctatus* throughout the active breeding-season for the species.

Little or no rain occurred for the first five days after spraying, and it has been shown that the 'Gammexane' during this dry period failed to penetrate the soil to a depth beyond 1 cm. from the surface, at least in a concentration toxic to fourth stage larvae of *C. impunctatus*. Thus, it may be said that, under the conditions of the experiment, 'Gammexane' in a miscible-oil base, applied in water to the breeding-grounds of *C. impunctatus* during a dry period at a dilution of 'Gammexane' of 1/500 to give a deposit of 100 mgm. 'Gammexane' per sq. ft., penetrates the soil to a depth of 1 cm. and results, for at least five days, in a percentage kill of the larvae present and either kills the pupae or prevents the subsequent emergence of adults from them.

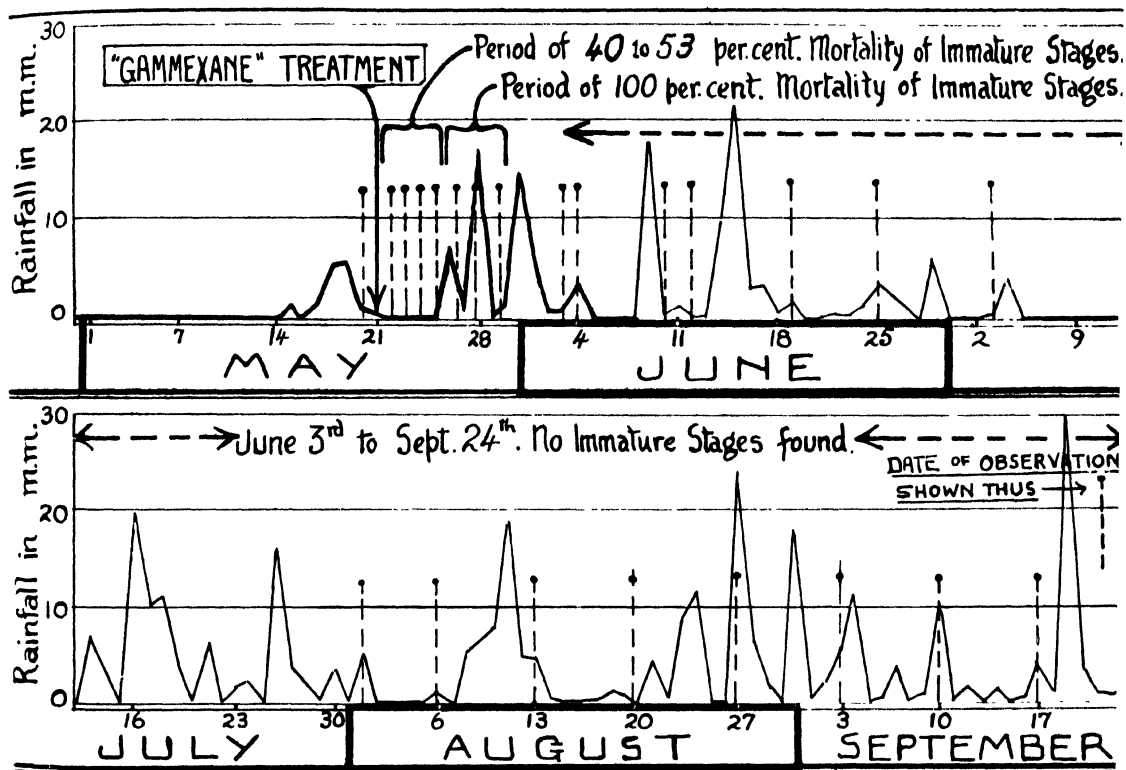


An interesting result, however, follows the downpour of rain (graph 4) on the night of the fifth day, in that, from the 'Gammexane' soil, only dead larvae or pupae were recovered within the next four days, and that thereafter, throughout a period of four months, no immature stages were found. This result contrasts sharply with the results for the control plots, which during the same period, as seen from graphs 1 and 2, show a marked seasonal increase in the larval density, substantially similar to that described for *C. impunctatus* under normal conditions by Hill (1947). Heavy rainfall might be expected to modify the effectiveness of the insecticide in two ways. First, it is probable, since 90 per cent. of the larvae occur in the top inch of the soil, that the deeper penetration would at first expose more larvae to contact with the 'Gammexane.' This would presumably give an increased mortality of larvae, particularly of those at the deeper levels, which might not normally approach the top soil until ready for pupation. The immediate consequence would be at least an increase in the proportion of dead larvae, or alternatively a complete kill, as is recorded in our observations. On the other hand, over such a long period of time as four months, the greater dispersal of the insecticide into the soil by rain could, in general, so reduce the concentration of 'Gammexane' at any one point as to render the insecticide partially or wholly ineffective. We have shown, however, that, despite heavy rain and waterlogging of the soil, the penetration of 'Gammexane' increased from 1 to 3 cm. only, and that there persisted a residual effect over the four-month period following the one application of the insecticide in May. Although this residual action was effective in preventing the survival of third or fourth stage larvae throughout the four-month period, the action was diminishing, as the time required to kill larvae increased from 24 hours immediately after spraying in May to seven days by September 17th (graph 5).

The factors causing this reduction in effectiveness over a period of time are probably complex and certainly obscure. Rainfall would appear to play little part in adversely affecting the residual action—indeed, it would seem to be a favourable factor, since, under the comparatively dry conditions prevailing in May at the time of the application of the insecticide, the first heavy rainfall appears to have promoted an increase in the effectiveness of the 'Gammexane' (graph 4). While it would be inappropriate to discuss, on the data available, other factors which may have been operative, it should be pointed out that the laboratory tests on the residual action were made on soil-samples taken from ground unshaded from the direct action of sunlight, whereas the samples taken to observe the control by 'Gammexane' of breeding under field conditions were collected from beneath the dense and low shade of rhododendron bushes where no direct sunlight could penetrate. This immediately imposes the limitation that any loss of residual action which may have occurred under conditions of shade may not, in fact, have been of the same order as was found to be the case in soil-samples from the unshaded area of the plot. Nevertheless, it seems legitimate to assume, if only by analogy with general knowledge concerning contact insecticides, that even in shaded soil a progressive reduction with time in the effectiveness of the residual action occurs.

We have shown that the residual action was effective in laboratory tests against third or fourth stage larvae of *C. impunctatus*, but it is not known which stage of the life-cycle under field conditions was sufficiently affected by the 'Gammexane' to cause the complete cessation of breeding which we observed in the field. Our observations reported below on the effects of 'Gammexane' on the adults of *Culicoides* spp. suggest that the concentration

of 'Gammexane' on the surface of the breeding-ground would have a limited effect—possibly not exceeding one or two weeks—on ovipositing adults, and that successful oviposition would, in all probability, be achieved by many midges before abnormalities of behaviour, or death, intervened. However, even assuming this to be true, it still remains obscure whether the 'Gammexane' exerts a direct ovidical action or whether it later kills the young larvae which hatch from the eggs.



GRAPH 5. Rainfall chart covering the period of the experiment, showing that a very dry spell of weather preceded the application of the 'Gammexane' on May 21st, and that, immediately after the treatment, a dry spell occurred until the evening of the fifth day, but that this was succeeded by frequent and heavy rain throughout the summer. The effects of the 'Gammexane' treatment on the immature stages of *C. impunctatus* with reference to the rainfall are indicated.

It is worthy of note that the last soil-samples were taken on September 24th, nearly a month after our last record for the occurrence of the adult midges (August 31st). In view of the fact that the eggs hatch within 14 days, it would appear that in the 'Gammexane'-treated plot in this experiment breeding was completely controlled throughout the active breeding-season of the adults by one application of the insecticide in late May. Since most of the adults for the summer of 1946 were due to emerge in June, it appears that the single treatment in late May destroyed not only the bulk of the adult population for 1946, but also the potential adult population for 1947, which, under normal conditions, would have been apparent as larvae in the soil of the treated plot from June onwards, at

a density comparable to that shown for the control plots and, after September, at a lower density as over-wintering larvae.

It may be said, therefore, that, under the conditions of the experiment described, 'Gammexane' in miscible oil applied once in water at a dilution of 'Gammexane' of 1/500 to give a deposit of 100 mgm. 'Gammexane' per sq. ft. on the breeding-grounds of *C. impunctatus* retains a residual action, probably diminishing but nevertheless effective, at least against third stage larvae for a period of not less than four months after the application. Furthermore, heavy rain within five days of the application of the insecticide results in an immediate improvement in the effects on the larvae and pupae, and frequent rain over a four-month period, to the extent of waterlogging the soil, does not lower the effectiveness of the residual action below a threshold level for killing larvae. If the insecticide be applied once, at the concentration used and at the time and under the conditions prevailing in the experiment, then not only is the bulk of the single summer generation of adults destroyed, but also the potential adult population for the following summer cannot develop in the treated soil.

## II. AN INVESTIGATION INTO THE EFFECTS OF 'GAMMEXANE,' IMPREGNATED ON BLACK CLOTH, ON ADULTS OF *Culicoides* MOMENTARILY IN CONTACT WITH THE CLOTH

*The General Plan of the Experiment and the Choice of Species.* Observations in Knowsley Park during the 1945 survey (Hill, 1947) had shown that large numbers of females of several species of *Culicoides* could be caught as they alighted and rested on pieces of black cloth suspended at a height of 4-5 ft. from the ground in a midge-infested area. On a still evening as many as 700 female midges had been observed in one hour on one side alone of a piece of black cloth 1½ ft. by 2 ft. in size. It was proposed to make this attraction of *Culicoides* adults to black cloth the basis of our experiment and to observe the effects of 'Gammexane' on midges momentarily alighting on cloths impregnated with known amounts of the insecticide. It was thought that such cloths once impregnated might, despite exposure for a long period to atmospheric conditions in the field, retain a residual action toxic to adults alighting on them, and that this might offer a means of controlling the adults of *Culicoides*. Of the species occurring in Knowsley Park, *C. impunctatus* and *C. obsoletus* were the most abundant and were in maximum numbers in June. Since both species were readily attracted to the black cloths it was convenient to make observations for each.

*Choice of Site.* A site where *C. impunctatus* and *C. obsoletus* occurred in large numbers has been described by Hill (1947), and a similar site under trees and bushes in Knowsley Park, Liverpool, known to harbour large numbers of both species, was chosen for the present experiment.

*Time of the Experiment.* The experiment was first set up in the field in June, during the period of maximum density for the adults, but the loss of the black cloths, owing to circumstances beyond our control, lead to this first experiment being abandoned. A second experiment was begun and completed in August, when the number of adults was less but still sufficient for our purpose. The actual collections of the midges from the black cloths were made in the evenings at approximately one hour before sunset, when *C. impunctatus* and *C. obsoletus* are most abundant.

*The Formulation and Concentrations of 'Gammexane' Used and the Method of its Application Against the Adults.* Again, as in the case of the experiment against the immature

stages, we had not traced any literature to guide us as to what constituted a suitable concentration of the insecticide for the control of the adults, and we therefore decided to test several concentrations. It was necessary to deposit known amounts of 'Gammexane' on pieces of black cloth, each  $1\frac{1}{2}$  ft. by 2 ft. in size ( $\equiv$  3 sq. ft. per side), and this was done by soaking pieces of black sateen of this size in solutions of a known weight of the pure

TABLE V

Showing that black sateen cloths, impregnated with 'Gammexane' at concentrations of 12.5-250 mgm. per sq. ft., become ineffective against *Culicoides* adults after about four weeks of exposure to weathering under field conditions, and cease to kill adults in appreciable numbers after the first two weeks

Date of test	Concentration of 'Gammexane,' in mgm. per sq. ft. of cloth	<i>C. impunctatus</i>		<i>C. obsoletus</i>		Total <i>Culicoides</i> adults	
		No. caught	Percentage dead after 15 hours	No. caught	Percentage dead after 15 hours	No. caught	Percentage dead after 15 hours
2.8.46	Nil (control)	20	0	22	4.5	42	2.27
1st day of exposure	12.5	18	100	16	87.5	34	94.12
	25	20	100	18	88.89	38	94.73
	50	16	93.75	24	91.66	40	93.75
	100	14	100	28	100	42	100
	250	20	100	26	100	46	100
8.8.46	Nil (control)	20	0	30	0	50	0
6th day of exposure	12.5	18	50	34	82.3	52	71.1
	25	24	75	30	73.3	54	74.07
	50	38	78.9	20	80	58	79.3
	100	24	100	26	80.8	50	90
	250	21	100	35	100	56	100
14.8.46	Nil (control)	20	0	30	6.66	50	4
12th day of exposure	12.5	24	66.6	26	53.8	50	60
	25	25	72	25	64	50	68
	50	21	52.38	29	72.07	50	64
	100	23	65.2	27	81.48	50	74
	250	22	77.27	28	64.28	50	70
20.8.46	Nil (control)	26	0	24	0	50	0
18th day of exposure	12.5	18	0	32	6.22	50	4
	25	23	4.34	27	0	50	2
	50	22	0	28	10.7	50	6
	100	20	20	30	16.6	50	20
	250	16	31.22	34	20.58	50	22
28.8.46	Nil (control)	5	0	20	5	25	4
26th day of exposure	12.5	12	0	40	0	52	0
	25	18	5.55	30	3.33	48	4.16
	50	21	0	25	0	46	0
	100	10	10	12	0	22	4.54
	250	21	9.5	4	0	25	8

crystalline gamma isomer of benzene hexachloride ( $\equiv$  'Gammexane') in benzene. Preliminary tests with benzene alone showed that 50 c.cm. of the solvent were required to give a thorough saturation of the cloth without excess of unabsorbed fluid, and that the midges did not appear to be affected by any residue from the benzene after its evaporation. The

cloths were rolled up lightly in a dish of the solution to ensure its even distribution and were then hung up to dry. By this method five portions of black sateen, each  $1\frac{1}{2}$  ft. by 2 ft. in size, were saturated with solutions in benzene of 75 mgm., 150 mgm., 300 mgm., 600 mgm., and 1,500 mgm. of crystalline 'Gammexane' to give deposits respectively of 12.5 mgm., 25 mgm., 50 mgm., 100 mgm., and 250 mgm. 'Gammexane' per sq. ft. of cloth. Since the insecticide was considered to have been evenly distributed on the cloth, the area over which it was deposited must be taken as at least 6 sq. ft. We have not endeavoured to make adjustments in the calculated deposit per sq. ft. of cloth to allow for absorption of the insecticide on the individual fibres, nor for that fraction lost to the dish in which saturation was carried out.

*The Method of Sampling and the Sequence of Observations Made on the Effects of the Deposits on the Adults.* The freshly treated cloths, together with a cloth treated only with benzene to serve as a control, were on August 2nd laid over a fence in the midge-infested area at a height of 4-5 ft. from the ground. Each *Culicoides* female as it alighted was caught by placing over it the open end of a test-tube, care being taken not to let the rim of the tube touch the cloth. The tube was then plugged with moist cotton wool. Collections were made for about 10 minutes from each cloth in turn, beginning with the control cloth and proceeding systematically through the series of cloths containing increasing deposits of 'Gammexane.' Although, at least on the first occasion, the midges were dying within half an hour of contact, it was convenient to examine the tubes on the following morning, approximately 15 hours after the insects had momentarily been in contact with a cloth, and to record then the percentage mortality of adults from each treated cloth and from the control cloth. This procedure was repeated with the same cloths at about six-day intervals on four occasions, namely, on the evenings of August 8th, 14th, 20th and 28th, and between these observations the cloths remained exposed to natural weathering by being suspended from the branches of trees at a height of 4-5 ft. from the ground.

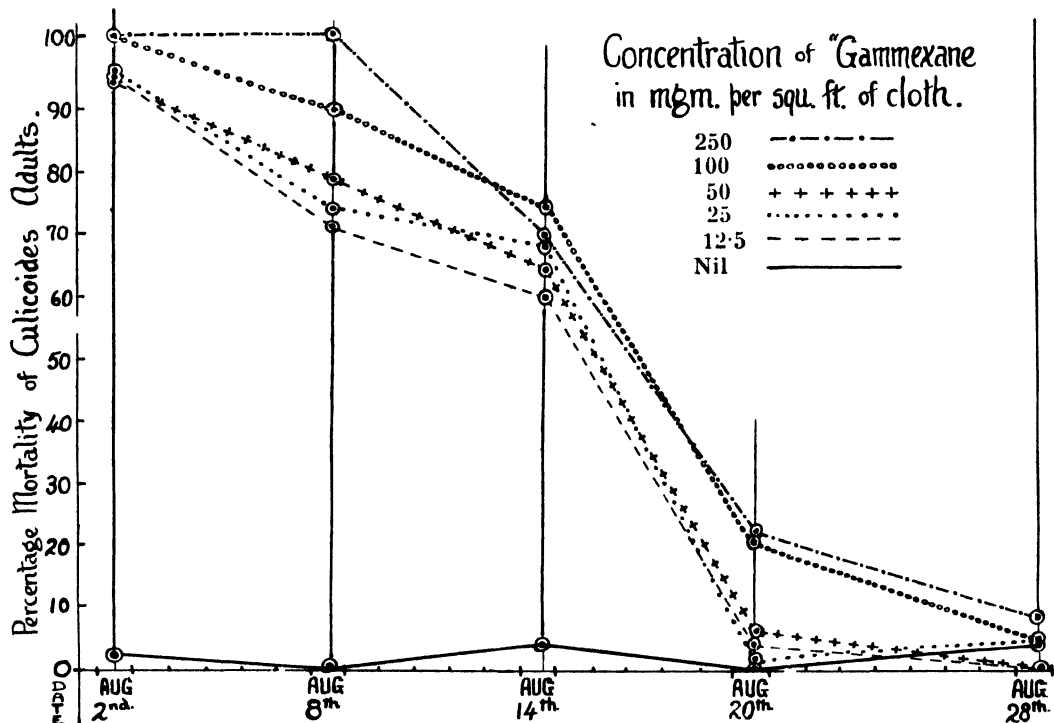
## RESULTS

### *The Effects of the 'Gammexane' on the Adults of C. impunctatus and C. obsoletus*

The results of the periodic collections and observations are recorded for each species in Table V, and, since there is no obvious reason why one species should react differently from the other to contact with the 'Gammexane,' the data for both are also compounded in the table and expressed diagrammatically in graph 6 to show the effects of 'Gammexane' on the adults of *Culicoides* as a whole.

It will be seen that when the cloths were first exposed on August 2nd the momentary contact of a midge with concentrations of 'Gammexane' varying from 12.5 to 250 mgm. per sq. ft. resulted, within 15 hours, in a mortality of 87.5-100 per cent. Of corresponding control midges not more than 5 per cent. died in the same period. After a period of six days of weathering, contact with the lower concentrations of 12.5-50 mgm. was effective in killing between 50 and 82.3 per cent. of the midges within 15 hours, but, in general, the higher concentrations of 100 and 250 mgm. per sq. ft. gave 80-100 per cent. kills in the same time. This was more apparent in the case of the most heavily impregnated cloth at 250 mgm. 'Gammexane' per sq. ft., which still retained a sufficiently effective residual action to kill 100 per cent. of both *C. impunctatus* and *C. obsoletus* adults. There was, however, a noticeable loss of residual action by all the deposits by the 12th day of the

exposure of the cloths, when the mortality varied from 52.4 to 81.5 per cent. Between the 12th and 26th days there was a continuous loss of effectiveness by all the concentrations of the insecticide, until, on the 18th and 26th days, a mortality of not more than 31.2 and 9.5 per cent. respectively of adult midges resulted within 15 hours after momentary contact with even the most heavily impregnated cloth. Throughout the series of tests, the mortality in adults from the control cloth never exceeded 6.7 per cent.



GRAPH 6. Showing that, of black cloths impregnated with 'Gammexane' and exposed for a month to weathering in the field, only the highest concentration of 250 mgm. insecticide per sq. ft. was completely effective in killing adult *Culicoides* (*C. impunctatus* and *C. obsoletus*) after about one week of exposure, and that a progressive loss of residual action followed, apparently at about the same rate, for all concentrations of the 'Gammexane,' until by the third or fourth week the effectiveness was negligible.

#### DISCUSSION

It is apparent that none of the deposits of 'Gammexane' on the black sateen investigated retains, under the conditions of the experiment, an appreciable residual action after about two weeks' exposure to weathering in the field. The cloth impregnated at 250 mgm. 'Gammexane' per sq. ft. was completely satisfactory for the first six days, and was the only treated fabric to give a 100 per cent. kill of all adults alighting on it at that time. The concentrations of 100 mgm. or less were similar in their effects after the first six days, except in the case of the cloth at 100 mgm., which gave a 100 per cent. kill of *C. impunctatus*, though only 80.8 per cent. for *C. obsoletus*. It is not surprising that the highest concentration gave such a consistently satisfactory result as opposed to the variable and

fluctuating results for the other cloths. The former contained at least  $1\frac{1}{2}$  times the concentration of 'Gammexane' on the other cloths, and of these all were within the relatively narrow limits of 12.5–100 mgm. 'Gammexane' per sq. ft. of cloth.

After the 12th day, apart from a slightly more effective residual action at 100 and 250 mgm. 'Gammexane' per sq. ft. of cloth, there is, as is shown in graph 6, little difference in the effectiveness of the cloths and some suggestion that the rate of loss of the residual effect is similar for all concentrations of the insecticide.

We are not prepared to discuss factors causing the observed loss of residual action, but it is certain that, whatever these may be, they would be expected to produce a variable effect, one way or the other, on the results obtained in this single experiment. Nevertheless, it would seem reasonable to conclude that black sateen cloths, impregnated with 'Gammexane' at concentrations of 250 mgm. or less per sq. ft. of cloth and subject to the field conditions imposed by the present experiment, are unlikely to prove a satisfactory method of controlling the adults of *Culicoides*, except possibly for a short interval of probably about two weeks, within a very restricted area.

### SUMMARY AND CONCLUSIONS

1. The literature concerning previous attempts to control the breeding of *Culicoides* species is reviewed.

2. An experiment on the control of breeding in *C. impunctatus* is described, in which a solution of 5 per cent. of the gamma isomer of benzene hexachloride ('Gammexane') in miscible oil is applied, at a dilution of 'Gammexane' of 1/500 with water, to give a deposit of 100 mgm. 'Gammexane' per sq. ft. of breeding-ground.

3. It was found that 'Gammexane' applied at this concentration under the comparatively dry weather conditions prevailing at the beginning of the experiment penetrates the soil to a depth of 1 cm., and results for at least five days in a mortality amongst the larvae of up to about 72 per cent., and either kills the pupae or prevents the emergence of adults from them.

4. It was further shown that heavy rain on the fifth day after the treatment increased the penetration of the insecticide to 3 cm. and resulted in an improved effectiveness, in that only dead larvae and pupa were obtained in the next four days and that no evidence of the breeding of *C. impunctatus* was found in the treated ground during the remainder of the normal breeding-season for the species. Despite persistent wet weather and the waterlogging of the soil throughout the summer, 'Gammexane' was never found to occur in a toxic concentration below a depth of 3 cm., and the residual action of the insecticide did not appear to have been adversely affected by the rainfall to any great extent.

5. It is shown that, within the conditions of the experiment, 'Gammexane' applied at a concentration of 100 mgm. per sq. ft. of breeding-ground of *C. impunctatus* in late May, before the bulk of the single summer generation of adults emerges, will not only destroy the generation due to emerge, but also prevents the development in the treated soil of the potential adult population for the following summer.

It is concluded from these results that, under the conditions present at the time of the experiment, the spraying of the breeding-grounds of *C. impunctatus* before adult emergence begins is an efficient method of controlling the pest.

6. An experiment designed to discover whether or not the attraction of female

*C. impunctatus* and *C. obsoletus* to black cloth could be used, by impregnating the cloth with 'Gammexane,' to control adult midges indicated that, although this method can result in the death of a high proportion of the midges alighting momentarily on the cloth, a heavy concentration of at least 250 mgm. 'Gammexane' per sq. ft. of cloth is required to ensure an effective residual action for as short a period as one week.

It is concluded from these results that, under the conditions prevailing at the time of the experiment, the exposure of black cloths impregnated with 'Gammexane' in midge-infested areas would have no wide-spread effect on the total population of *Culicoides* adults.

ACKNOWLEDGEMENTS.—We are greatly indebted to the Right Honourable the Earl of Derby, K.G., for allowing the experiments to be carried out on his estate at Knowsley Park, Liverpool, and to Mr. F. A. Long, Estate Agent to Lord Derby, for his unfailing courtesy and co-operation. We also wish to thank Lieutenant-Colonel R. W. Stephens, M.B.E., for his kindness in assisting us in several aspects of the work. We are indebted to the Director, Liverpool Observatory and Tidal Institute, for the rainfall records.

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# NEMATODES IN TSETSE

BY

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In the course of the dissection of some 1,500 *Glossina morsitans*, three examples of a nematode worm of the *Mermis* type have recently been found; their average length was 79 mm. These parasites were all found during the wet season, as were those noted by the Scientific Mission of Katanga (Rodhain *et al.*, 1913). Similar worms of the *Mermis* type have also been recorded by Lloyd (1912)—again in the wet seasons—from *G. morsitans* in the Mpika area of Northern Rhodesia, 200–300 miles from Abercorn, and by Minchin (Lloyd, 1912) and Carpenter (1912, 1913) from *G. palpalis* on Lake Victoria in Uganda.

I am indebted to Dr. H. A. Baylis for a still earlier reference to the occurrence of *Mermis* in *G. palpalis* than those already cited—that of Leiper (1910). Here again there is no description beyond the statement that the worm was an immature female, 3 in. long. The locality where it was found was Entebbe.

The worms now recorded appear to be somewhat larger than those previously observed, and seemed to occupy so much of the abdominal cavity as seriously to incommode the tsetse; thus in one specimen caught there were samples of two different bloods, each with recognizable corpuscles, as if the tsetse had been unable to take up enough blood at one time to satisfy its needs, and had been forced to take two meals within an unusually short time. Even then the fly was still hungry enough to attack the collectors, and its fat-contents were those of a hungry fly.

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# PHOTOSENSITIZATION OF ZEBU CATTLE ASSOCIATED WITH THE ADMINISTRATION OF PHENANTHRIDINIUM 1553

BY

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It has recently been shown that phenanthridinium compounds (Morgan and Walls, 1938) are of great value in the treatment of trypanosome infection in cattle (Hornby *et al.*, 1943; Carmichael and Bell, 1944*a*, 1944*b*).

During the course of later experiments to investigate the therapeutic value of phenanthridinium 1553 (2 : 7-diamino-9-phenyl-10-methylphenanthridinium bromide), the drug was given intravenously to a group of cattle in order to ascertain the maximum tolerated dose. It was noted in this latter investigation that, following the injection of relatively large amounts of phenanthridinium 1553, the cattle showed delayed toxic symptoms, including photosensitization (Bell, 1945). The present article gives some account of this toxic effect of the drug on cattle.

## HISTORY OF THE CATTLE

The cattle were part of a group of healthy mature Zebus which had been infected with *Trypanosoma congolense* and had been treated and cured by phenanthridinium 1553 when they were in the chronic stage of the disease. The amount of 1553 which they received varied from 0.5 mgm./kgm. to 1.0 mgm./kgm. live weight. When it had been established that the *T. congolense* infection had been cured the animals were turned out to grass.

Some months later the animals were used to ascertain the maximum tolerated dose of the phenanthridinium compound 1553. They received varying dosages of a freshly prepared 2 per cent. solution of phenanthridinium 1553 ranging from 3.0 mgm. to 6.0 mgm./kgm. body-weight intravenously.

The reaction to this treatment varied : a dose of phenanthridinium 1553 equivalent to 3.0 mgm./kgm. body-weight had no toxic effects, 4.0 mgm./kgm. produced mild toxic effects, 5.0 mgm./kgm. produced marked toxic effects. Of the four animals that received 6.0 mgm./kgm. two died immediately. The toxic effects of the drug were of a generalized depressant type, except in one animal where the action appeared to be excitatory.

After the completion of the experiment the survivors were kept under observation for two days before they were turned out to graze alongside another large herd of cattle. After 45 days it was reported that the animals were sick, and they were then brought to a small paddock close to the laboratory for observation.

## SYMPTOMS

It was obvious that, of the 11 head of cattle which had received the phenanthridinium 1553, 10 were suffering from photosensitization ; two of them died the same day. One

animal, however, no. 5098, showed no signs of photosensitization and remained healthy and normal in every way.

The general symptoms shown by the remaining eight animals were :

1. Extreme emaciation, with hidebound and staring coat.

2. General weakness, with incoordinated movement.

3. Marked restlessness, head-shaking, licking of anus and flanks, stamping of the feet, rubbing against obstacles, photophobia—the animals invariably sought shade during the day-time, even the scantiest cover, such as the shade of a timber fence.

4. Inappetence, grinding of teeth, cessation of rumination, constipation. Faeces hard, scanty, black in colour.

As soon as it was noticed that the animals were sick a record was made of their morning and evening temperatures (see chart). The season at the time of the observations was extremely hot, with an estimated shade temperature of 75° F. in the morning and late evening and up to 85° F. in the early afternoon. The outside atmospheric temperature would, of course, be greatly in excess of these figures. Two animals (5051, 5143) were stabled, and the temperature-curve gradually settled after a few days, although one animal (5051) died in spite of the protection.

1. No. 5280. Bullock, colour red. Age 4 years. Received 4.0 mgm./kgm. 1553. Face swollen, especially around muzzle and eyes. Serum oozing from inner canthus of eye, muzzle, base of ears, base of horns. Base of horns swollen and horn 'flaking off.' Both surfaces of ears oozing serum, which dried to form crusts. Buccal mucosa and conjunctiva hyperaemic and jaundiced. Intermandibular oedema extending down to the dewlap. Severe irritation at horn bases, which caused the animal to scratch with its hind legs. Destroyed *in extremis* 49 days after receiving 1553.

2. No. 5170. Bullock, colour grey. Age 4 years. Received 5 mgm./kgm. 1553. Muzzle slightly swollen and inflamed. Eyelids swollen. Muzzle dry and scaling, with small discrete pockets of pus beneath the epidermis. Mucous nasal discharge. Conjunctiva icteric. Skin jaundiced, especially at the base of the tail and perineal region. Destroyed *in extremis* 50 days after receiving 1553.

3. No. 5084. Bullock, colour grey. Age 3½ years. Received 6.0 mgm./kgm. 1553. Muzzle normal, buccal mucosa swollen and slightly hyperaemic. Conjunctiva hyperaemic and jaundiced, with serum oozing from margin of lower eyelid. On the ventral aspect of the ears there were beads of dried exudate and serum. Peri-anal mucosa yellow in colour, with exudation of serum. Skin hard and tumified over back. Extremely restive. Ears became very much swollen. Serum oozing from back and flanks. Became very weak; recumbent. Destroyed *in extremis* 52 days after receiving 1553.

4. No. 5123. Bullock, colour red and white. Age 5 years. Received 5 mgm./kgm. 1553. Face swollen. Papillae at commissures of the mouth inflamed. Intermandibular oedema. Conjunctiva jaundiced, sero-purulent discharge from the inner canthus. Ears thickened and swollen, with dried serum incrustations at their bases and extending down the neck. Skin thickened and covered with scabs; dewlap thickened. Irritation became intense. The animal became progressively weaker, with stiff gait, gross oedematous swelling of the head, and shedding of epidermis at the root of the tail. Destroyed *in extremis* 54 days after receiving 1553.

5. No. 5249. Bullock, colour dark brown. Age 3 years. Received 5 mgm./kgm. 1553. Muzzle epithelium desquamating, with old crusts and oozing serum. Buccal

mucosa swollen, hyperaemic and jaundiced. Muco-purulent nasal discharge. Eyes sunken, conjunctiva hyperaemic. Necrotic ulcers with pus at inner canthii, nictitating membrane and edges of lower eyelid, the necrosis extending into the eye-socket and involving the conjunctiva. Ears swollen and oozing serum, edges necrotic and contracted. The animal became progressively weaker and was destroyed 57 days after receiving 1553.

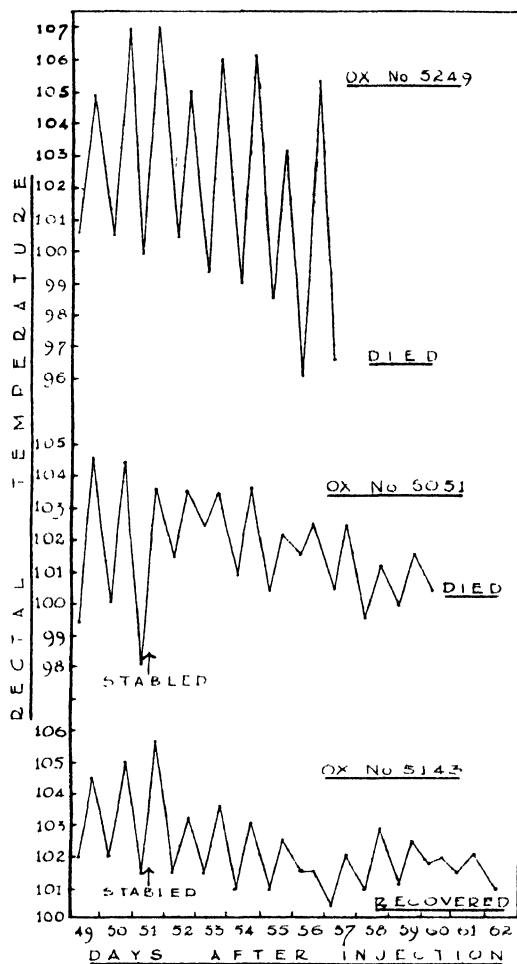


CHART. Temperature-curves showing the effects of housing the animals. Nos. 5051 and 5143 were stabled 51 days after injection; no. 5249 was left in the open.

6. No. 5051. Bullock, colour red and white. Age 3 years. Received 5 mgm./kgm. 1553. Muzzle swollen and oozing serum; tessellation and pores distinct. Serous nasal discharge. Serum oozing from ears and base of horns. Ears swollen and exuding serum, which later formed scabs which peeled off, leaving raw surface. Ears necrotic at edges, causing contraction and curling. Eyelids swollen, conjunctiva hyperaemic and jaundiced. Buccal mucosa swollen and icteric. Eye discharge increased, and eyes became slightly

shrunk. This animal was stabled on the third day of observation, its temperature reaction settled down (see chart), but it became recumbent and was destroyed 60 days after receiving 1553.

7. No. 5068. Heifer, colour grey. Age 3 years. Received 3 mgm./kgm. 1553. Tessellation of muzzle very distinct, oozing serum. Conjunctiva yellow in colour. Vulva and anus hyperaemic and swollen. Hair on tuber ischii removed by licking; skin yellow. Vulva icteric; whole of perineal region oedematous. This animal retained its appetite, the faeces remained normal, and it eventually recovered.

8. No. 5143. Bullock, colour grey. Age 3 years. Received 4 mgm./kgm. 1553. Muffle and lower lip very jaundiced, with necrotic areas. Muzzle slightly swollen. Conjunctiva hyperaemic and swollen. Ears dry and contracted at the edges. Anal skin jaundiced and hyperaemic. The root of the tail became necrotic and gangrenous; some of the epidermis was shed. The dewlap became thickened and hard; later, patches of epidermis sloughed. The epidermis sloughed from the axillae and the flanks, tuber coxae and tail, leaving a yellow surface. The animal was stabled and recovered.

#### NATURE OF THE BLOOD SERA

When it was noticed that jaundice was a fairly constant symptom, blood was drawn from the jugular vein and allowed to clot, and the serum was drawn off. The macroscopic appearances of the sera were as follows:

Normal colour	...	...	...	...	5051, 5068
Slight increase in yellow colouring	...	...	...	...	5170, 5143, 5123
Light brown	...	...	...	...	5084
Brown-red	...	...	...	...	5280
Dark red	...	...	...	...	5249

Conditions did not permit of chemical examination.

#### MORBID ANATOMY

*Flesh and Subcutaneous Tissues.* Flesh, boiled appearance and yellowish in colour. Subcutaneous oedema, fluid bright yellow in colour, gelatinous. Subcutaneous vessels dilated, producing a cobweb effect.

*Serous Cavities.* Peritoneum thickened, opaque, yellow in colour. Petechiae on omentum. Some increase of peritoneal fluid, but never marked. Pleura and pericardium thickened, opaque yellow in colour.

*Buccal Cavity, Pharynx and Oesophagus.* Mucosa yellow in colour.

*Stomach.* Submucosa of abomasum lamellae infiltrated with fluid. Some erosion of mucosa. (N.B. Gutzeit test for arsenic negative.) Rumenal contents dry.

*Small Intestine.* Mucous membrane pale and slightly wrinkled. Contents normal.

*Large Intestine.* Mucous membrane slightly jaundiced and thickened. Rectal mucosa thickened and tinged yellow. Contents dehydrated.

*Liver.* Swollen, with rounded edges. Capsule thickened and tough. Parenchyma yellow-brown in colour, tough to the touch, lobulation distinct. Gall-bladder distended, bile normal. Mucosa of gall-bladder with many bright red ecchymoses (5 mm. in diameter). Some *Fasciola* spp. present.

*Central Nervous System.* Brain, boiled appearance. Meningeal vessels dilated.

*Nasal Cavity, Larynx and Trachea.* Submucosal vessels dilated. Mucous membrane stained yellow.



FIG. 1. Ox 5051. Liver, picro-Mallory,  $\times 56.5$ . Showing peripheral congestion, dilatation of central veins, and necrosis of liver-parenchyma.

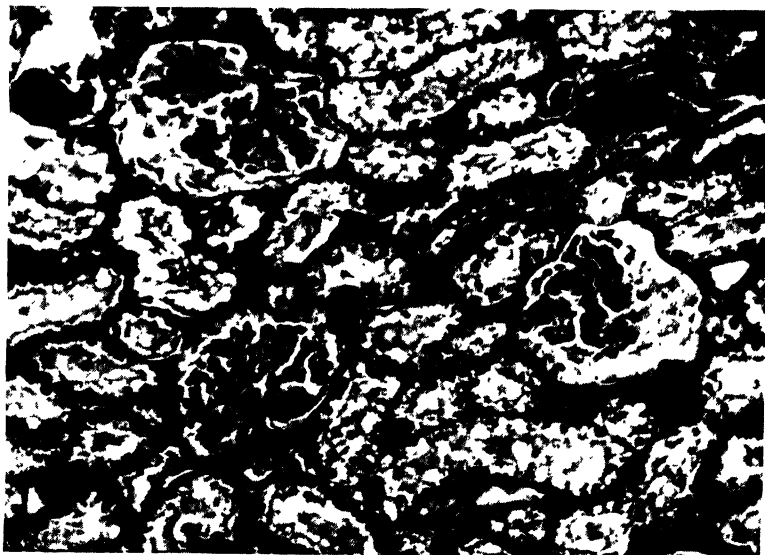


FIG. 2. Ox 5051. Kidney, picro-Mallory,  $\times 200$ . Showing degeneration of epithelium and contraction of glomeruli, with haemorrhage into capsule.



FIG. 3. Ox 5213. Spleen, stained for iron, eosin counterstain,  $\times 50$ . Showing massive deposits of iron-containing pigment.

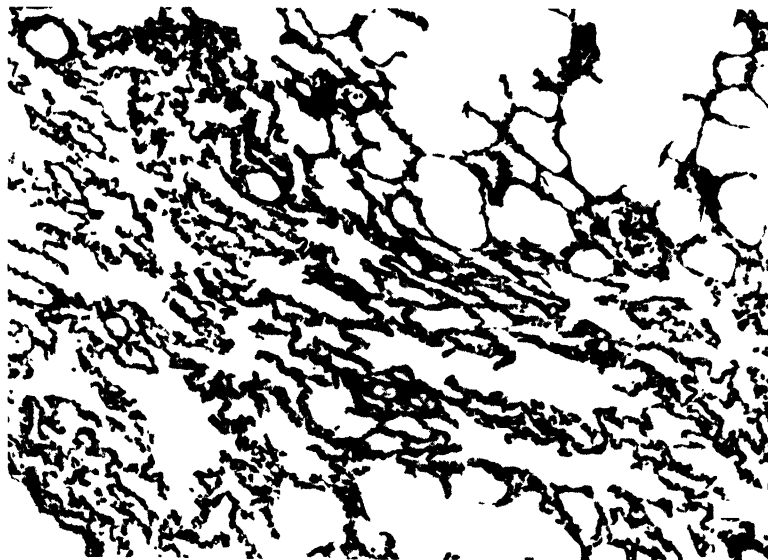


FIG. 4. Ox 5280. Lung, H. and V.G.,  $\times 145$ . Showing collapse and compensatory emphysema.

**Bronchii and Lungs.** Lungs collapsed but float in water, some depressions on surface, brick-red in colour. Interstitial emphysema. In two cases (5261, 5244) lungs distended. Visceral pleura thickened, yellow, opaque.

**Heart and Blood-Vessels.** Hydropericardium (yellow fluid). Pericardium thick and dull appearance, yellow. Heart dilated, especially right ventricle. Areas of haemorrhage on papillary muscles of right ventricle. Coronary fat and auriculo-ventricular valves stained deep yellow. Some *Cysticercus bovis* present.

**Spleen and Lymphatics.** Spleen capsule thickened, tough, yellow. Normal size. Pulp tough to touch. Lymph-glands slightly swollen and oedematous.

**Kidneys.** Perirenal fat yellow in colour, gelatinous. Capsule strips easily, parenchyma hard and pale, bright yellow in colour. Mucosa of calyces bright yellow in colour.

**Bladder.** Urine dark brown. Mucous membrane yellow.

### MICROSCOPIC EXAMINATION

**Liver** (Plate V, fig. 1). Congestion, with central lobular vein and sinusoids dilated. Cloudy swelling, fat infiltration and necrosis of liver-cells. Kupffer cells very active. Marked increase in number of bile-ducts in portal tracts. Pigment scattered throughout organ. Potassium ferrocyanide demonstrated scanty deposition of iron in some sections only.

**Kidney** (Plate V, fig. 2). Glomeruli contracted, with haemorrhage into capsule. Cloudy swelling and necrosis of renal epithelium, most marked in cortex; cellular debris blocking lumen of tubules.

**Spleen** (Plate VI, fig. 3). Malpighian bodies very active. Sinusoids congested. Pigment in excessive amounts in red pulp of all sections. Potassium ferrocyanide demonstrated presence of large amounts of iron-containing pigment.

**Lymph-Gland.** Congested and active.

**Lung** (Plate VI, fig. 4). Congested. Lobular collapse and emphysema in all sections, but varying in extent in different animals.

**Adrenal Gland.** Congested.

**Thyroid, Pancreas, Pituitary, Brain, Skeletal Muscle and Heart-Muscle.** Appeared normal.

### DISCUSSION

The symptoms produced in cattle following the injection of relatively large amounts of phenanthridinium 1553 closely simulates the condition of small stock in South Africa known as 'geeldikkop' (Theiler, 1918), jaundice, subcutaneous oedema and photosensitization being common to both conditions. Following surgical obstruction of the normal bile-flow of sheep and goats, Quin (1933*b*) describes a chain of symptoms similar to those described above, viz., clinical icterus, marked emaciation and photosensitization. Photosensitization and icterus have been reported in congenital porphyrinuria of cattle (Fourie, 1936).

In the condition under review, however, the onset of photosensitization is very much delayed, being on the average 50 days after the injection of the drug. This is in contrast to the very rapid photosensitizing action of other photodynamic compounds, such as the fluorescent dyes, when an induction-period of only a few hours is required (Quin, 1933*a*). It is probable that the phenanthridinium compounds are excreted from the body



fairly rapidly, since they exert a very short protective action against *T. congolense* infections in cattle (Carmichael and Bell, 1944a). Brownlee (1946) has shown that the major part of a dose of phenanthridinium 1553 administered to rabbits is eliminated from the body in a few days. In view of these findings it is possible that the phenanthridinium 1553 is not in itself the photodynamic agent, although it is perhaps associated with the genesis of some such agent. It is possible that some degradation product of the drug may be the photodynamic agent.

Phenanthridinium 1553 is associated with photosensitization only when large amounts are administered, which produce extensive damage of the parenchymatous organs and especially of the liver. Such extensive damage of the liver would interfere with erythropoiesis and would possibly cause the production of the photodynamic members of the porphyrin group of pigments (cf. Rimington, 1936; Brownlee, 1939). Furthermore, the damage to both liver and kidneys would interfere with the excretion of other pigments by these organs, so that the serum bilirubin would accumulate to produce symptoms of jaundice. Coincidentally, any photodynamic pigment (coproporphyrin) would also accumulate until it reached the required threshold-level necessary to induce photosensitization.

Wien (1946) has produced evidence that phenanthridinium 1553 administered to experimental animals produces liver damage, characterized by fatty degeneration, but no porphyrinuria. Brownlee (1946) has confirmed this finding, and states that treated animals irradiated with ultra-violet light show no symptoms of photosensitization. It should be noted that in both these experiments the experimental animals were either sacrificed or died within 28 days of the administration of the drug.

The cattle showing photosensitization described above had all been previously infected with *T. congolense*, and, although they had been cured of this infection, it is possible that some initial lesion due to *T. congolense* is of importance in conditioning the liver to the effects of phenanthridinium 1553. Roets (1938) has reported that another protozoan infection of cattle, East Coast fever, does produce a relative increase in coproporphyrin I.

The effects of phenanthridinium 1553 on respiration are interesting, for histological examination of the lung shows very extensive areas of collapse. In the original experiments the depressant effect of the drug on respiration was noted (Bell, 1945). Wien (1946) suggests that this deleterious effect is due to the method of administration, since intrajugular injection would produce a sudden high concentration in the lungs.

The fact that phenanthridinium 1553 in amounts well in excess of the therapeutic dose is associated with photosensitization should not detract from its value as an extremely active trypanocidal drug against the monomorphic trypanosomes. It has been shown that in routine treatment of *T. congolense* and *T. vivax* the drug is very effective and does not produce symptoms of jaundice or photosensitization (Stewart, 1946; Barnett, 1946).

The therapeutic index for phenanthridinium 1553 of 6.25 computed by Bell (1945) was calculated by using a figure for the maximum tolerated dose which is immediately fatal for cattle, and which therefore does not take into account the delayed toxic effects when animals surviving this dosage are exposed to extreme solar radiation. Taking into account this delayed toxic effect, the therapeutic index would be reduced to 5.0; but in view of the abatement of photosensitization phenomena in the animals that were stabled, and by analogy with other similar cases, it is possible that, if all the animals had been housed immediately they showed symptoms, they would have survived, and the

original computation would remain correct. The fact that the animals used to ascertain the maximum tolerated dose were not normal, in that they had previously been infected with trypanosomiasis and cured with a therapeutic dose of phenanthridinium 1553, must also be recognized.

In the use of phenanthridinium 1553 in the field the possibility of photosensitization should be looked for, and whenever possible the animals should be protected from direct sunlight or treated in seasons of the year when solar radiation is at a minimum, as has been advised in the immunization of sheep against blue-tongue virus (Neitz and Riemerschmid, 1944). Where it is impossible to house all the animals, or when it is necessary to treat them during the hot season, any animal showing loss of condition or jaundice should be housed immediately.

### SUMMARY

1. Phenanthridinium 1553 administered to cattle intravenously in doses ranging from 3 mgm. to 6 mgm. per kgm. live weight may produce delayed toxic symptoms and death after 49-60 days.

2. The symptoms are extreme emaciation, jaundice, photophobia and dysfunction of the thermo-regulatory mechanism.

3. A marked toxic effect of the drug on the parenchymatous organs is demonstrated.

4. The possible ways wherein the drug can be associated with the onset of photosensitization are discussed.

5. The relationship of the toxic symptoms, the therapeutic index and the field-use of the drug is discussed.

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# OBSERVATIONS ON THE LIFE-CYCLE OF *SCHISTOSOMA MANSONI* IN THE LABORATORY, WITH A DISCUSSION ON THE SNAIL VECTORS OF *S. MANSONI* AND *S. HAEMATOBIIUM*

BY

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Certain points of interest were noted following the establishment of a strain of schistosomiasis in the laboratory, which was undertaken in Liverpool at the request of the Medical Research Council.

The initial step of obtaining and establishing a supply of suitable molluscan vectors in this country has already been described (Cowper, 1946). The vectors used were *Bulinus truncatus* and *Planorbis boissyi*, laboratory-bred from Egyptian stock, and *Australorbis glabratus* (= *Planorbis guadaloupensis*), bred from stock brought to England from the laboratories of the I.G. Farben Werke in Elberfeld, through the kind assistance of Dr. W. Kikuth.

The complicated life-history, the need to maintain colonies of tropical snails, and the scarcity of clinical cases passing viable ova outside the endemic areas tend to make the establishment of a strain of *Schistosoma mansoni* and *S. haematobium* in a temperate country more difficult than is the case with many other parasitic infections. It has, however, been established and maintained before at several centres in London, Germany and the United States of America. *S. mansoni* is easier to maintain than *S. haematobium*, and the *Planorbis* snails seem to be easier to breed and maintain than *B. truncatus*. There is much evidence to show that the tropical American *A. glabratus* is the most suitable vector of *S. mansoni* for laboratory work.

## THE EXPOSURE OF *P. BOISSYI* AND *A. GLABRATUS* TO MIRACIDIA FROM A UGANDA STRAIN OF *S. MANSONI*

A number of exposures of *A. glabratus* and *P. boissyi* to miracidia of *S. mansoni* were carried out. The miracidia were obtained from ova passed by two patients, infected in Uganda and admitted to a Liverpool hospital under the care of Dr. A. R. D. Adams. Miracidia were obtained following concentration of ova by filtration of the stools through gauze, centrifugation, and finally maintenance of the filtered and concentrated faeces in tap-water in Petri dishes at a constant temperature of 23–24° C. in an insectarium until active miracidia were observed. The snails were exposed over night to miracidia in Petri dishes, and, following exposure, were then transferred back to tanks kept in a heated insectarium, which gave an average water-temperature of 21·5–23° C. The snails were always handled with a tea-spoon to avoid crush injuries. In the case of *P. boissyi* actual penetration by miracidia was observed. Examination of the snails for cercariae was commenced one month after exposure to miracidia. The snails were generally placed for a period of 8–36 hours in small pots containing rain-water, in case the chlorine content of Liverpool tap-water might be toxic to cercariae, and were exposed to sunlight, an artificial-daylight lamp or a bright bench-lamp. The pots were then

examined with a hand-lens for cercariae. About six weeks after exposure several *P. boissyi* died, but when dissected they showed no sign of sporocysts or cercariae.

Between 48 and 63 days after exposure moderate numbers of cercariae were shed by one of the *P. boissyi*.

A monkey was exposed to approximately 250–300 cercariae applied to the shaved skin of the belly and to the lips and mouth. The cercariae were enumerated by the following simple technique: a small Petri dish was divided into four quadrants with a coloured pencil, placed on a piece of black paper and filled to the brim with water containing the cercariae. The cercariae, showing silvery-white against the black background, were counted in each quadrant with a hand-lens. The Petri dish was then used as a measure of the number of cercariae given to the monkey.

A comparatively small number of cercariae were shed by one of the *A. glabratus* between 56 and 59 days after exposure. A few degenerating sporocysts, but no cercariae, were found in dead *A. glabratus*. It thus appears that both *P. boissyi* of Egyptian stock and *A. glabratus* of (presumed) South American or West Indian stock are potential vectors of a Uganda strain of *S. mansoni*.

The period from exposure of the snails to miracidia up to the appearance of the first cercariae was 48–59 days under the conditions described above. It is known that this incubation-period varies with temperature and sunlight, and numerous investigators—in particular, Blacklock and Thompson (1924) and Gordon, Davey and Peaston (1934)—have investigated the variations in incubation-rate in tropical climates. In laboratories in temperate climates periods varying from four to eight weeks are recorded.

The monkey exposed to cercariae began to pass ova just four months after exposure, and a strain of *S. mansoni* was thus established.

#### EXPOSURE OF EGYPTIAN *B. TRUNCATUS* TO MIRACIDIA FROM A WEST AFRICAN STRAIN OF *S. HAEMATOBIMUM*

Adult and half-grown specimens of *B. truncatus* bred in the laboratory from an original Egyptian stock were exposed to miracidia of *S. haematobium*. The ova were obtained from a patient in a Liverpool hospital who had become infected in Nigeria, where the normal vector is *Physopsis globosa*.

Ova were successfully preserved viable in normal saline in an ice-chest for at least two or three days. When miracidia were required the ova were washed by centrifugation, to remove urine, and kept in tap-water in watch-glasses at a temperature of 23–25° C. in the tropical insectarium. Active miracidia were normally obtained within 24 hours, and often in much less time. Exposure of the snails was then effected by one of two methods, as follows:

1. Snails were given a short exposure of a few minutes to a high concentration of miracidia in a watch-glass at laboratory temperature.
2. Snails were exposed during the afternoon and over night at a temperature of 23–25° C. to a thinner concentration of miracidia in a Petri dish.

Altogether 22 snails were exposed by these methods.

The snails were handled with a tea-spoon, and preference was given to half-grown specimens. Examination for cercariae was commenced one month after exposure and continued on most days until all the snails were dead, two months after exposure. The snails were placed in small pots or jars in aquarium-water and exposed to direct sunlight

or to a bright bench-lamp. The water was then examined with a hand-lens for cercariae. Snails which died were dissected and examined for sporocysts or cercariae, but in no case were they found to be infected.

Throughout the whole period no sign of cercariae was observed, and it was concluded that penetration and development had not taken place. This corroborated observations made on the snails in watch-glasses during the exposure to miracidia. The latter were observed cruising round the snails, passing under the shell or mantle, and sometimes fortuitously striking the soft parts, including the tentacles; but in no case was penetration observed or anything which could be described as a definite attraction.

It was concluded that Egyptian *B. truncatus* do not attract miracidia from a West African strain of *S. haematobium*.

### DISCUSSION

These experiments are recorded as a minor contribution to the whole question of the specific relationship between the snail and the miracidium. In the last 30 years an accumulation of evidence relative to the snail vectors of schistosomes in many parts of the world, as well as numerous laboratory investigations, appears to indicate not only that the vector species of snail varies in different parts of the world, but that miracidia from a certain local strain of schistosome are attracted mainly or only by the particular local snail vector. Not only a different species of vector, but a different strain of the same species of snail may prove non-attractive.

In unpublished notes Stunkard has described his failure to infect Egyptian *P. boissyi* with a Puerto Rican strain of *S. mansoni* in New York. Since *S. mansoni* may have reached the New World with West African slaves, a West African *Planorbis*, such as *P. pfeifferi*, might prove more attractive. The existence of a refractory and a susceptible strain of *P. boissyi* in the Nile Valley has already been suggested by Barber. Studies on American snails have resulted in Cram and Files (1945) successfully passing *S. mansoni* through a Louisiana species of *Tropicorbis* and producing cercariae, but other North American Planorbidae were refractory. The experiments described above show that a Uganda strain of *S. mansoni* could complete its development both in Egyptian *P. boissyi* and in the tropical American species *A. glabratus*.

With regard to *S. haematobium* and *Bulinus*, the above experiments would seem to indicate that the Egyptian *B. truncatus* (now considered as synonymous with *B. dybowskii*, *B. innesi* and *B. contortus*) did not attract miracidia of a Nigerian strain of *S. haematobium*. In Nigeria and elsewhere in West Africa the vector is *Physopsis globosa*. Blacklock (1925) describes a *P. globosa*-like carrier in Sierra Leone, while Blackie (1932) describes *P. globosa* and *P. pfeifferi* as the accepted vectors of *S. haematobium* and *S. mansoni* respectively in Southern Rhodesia. Chesterman (1923) describes *Physopsis africana* as the vector in the Belgian Congo, where terminal spined ova appear to be frequent in the faeces. Adams (1934), following a full and detailed investigation of the transmission of schistosomiasis in Mauritius, found that *Bulinus (Pyrgophysa) forskali*, alone of the various snails which he exposed, showed a powerful attraction for miracidia from Mauritian cases of *S. haematobium*. *B. forskali* is widespread in Africa and occurs in Egypt and South Africa, though apparently it has not been incriminated as a vector there. Leiper (1915) indicated that in Egypt *B. (P.) forskali* showed attraction for miracidia, but he did not list it as a definite vector. Manson-Bahr and Fairley (1920), also in Egypt, failed to infect *Physa*

*subtropica*, and, while listing *B. contortus*, *B. dybowskii* and *B. innesi* (now united as *B. truncatus*) as vectors and mentioning the occurrence of *B. forskalii*, they did not apparently consider the latter as a vector. Adams thinks that it is probably the only vector on the island of Mauritius; it is certainly the most important. Porter (1920) claimed to have found the cercariae of *S. mansoni* in *Physopsis africana* and in a (then) new host, *Planorbis pfeifferi*, now established as the chief *mansoni* vector in West and Central Africa. This *Ph. africana* belongs to the genus which is elsewhere the vector of *S. haematobium*, just as in Portugal and Morocco a *Planorbis* (*P. dufourii*) has become the vector of *S. haematobium*, while elsewhere only *S. mansoni* is transmitted by the genus *Planorbis*.

Leiper again, in 1928, has called attention to the interesting case of the village of Syrianokhori in Cyprus, where for many years *S. haematobium* has been endemic, while *B. contortus* (i.e., *B. truncatus*) was found by him in the neighbourhood, together with *Melanopsis* sp. This appeared to be the only focus of schistosomiasis in Cyprus. Buchanan (1937) considers *Melanoides tuberculata* the probable vector in the Berber region of the Sudan, in addition to *B. truncatus* described as a vector in this area by Archibald. Buchanan further quotes Gopshill as authority for describing *M. tuberculata* as a vector in Nyasaland. The recorded vectors of *S. mansoni* are fewer in number—pre-eminently *P. pfeifferi* in Equatorial Africa, *P. boissyi* in the Nile valley, and *A. glabratus* and possibly other species in tropical America. But here again other vectors may be incriminated locally; Sautet and Marneffe (1944), for example, reported *Planorbis adowensis* as a vector in the French Sudan.

There seems to be a puzzling problem here: why is it that in one locality a species of schistosome can adapt itself even to a different genus of snail vector, while elsewhere the correct normal vector of one locality proves unattractive to the miracidia of a strain of the same schistosome species from another locality? It would seem that schistosomiasis in any one area is closely linked not only with one species of snail vector but with the local strain of that species, and that another strain of the same snail from elsewhere may prove refractory. It would be instructive to repeat the above experiment with *Bulinus* and *S. haematobium* in the reverse direction, i.e., to see whether West African *Physopsis* attracted miracidia from an Egyptian case of *S. haematobium*. Obviously the whole question must be of importance in relation to 'species sanitation' in any snail-control campaign and emphasizes the necessity for establishing the local vector on the spot in any given locality. Furthermore, it increases the already complex difficulties of establishing strains of schistosomiasis in the laboratory for experimental work outside the tropics; to do so requires, ideally, that both infected definitive hosts and the local snail vector should be brought together from the endemic area. On the other hand, this high degree of local snail-miracidium specificity may be one reason why (apart from the case of *S. mansoni* in tropical America, where *A. glabratus* has apparently proved an optimum vector) the spread of schistosomiasis outside Africa has been very limited. Likewise the danger of introduction of the infection into India or Burma by means of West African soldiers might, therefore, be less than has been surmised.

### SUMMARY

1. Cercariae of *Schistosoma mansoni* were obtained from *Planorbis boissyi* of Egyptian stock seven and a half weeks after exposure to miracidia from a Uganda strain of *S. mansoni*. A few cercariae were also obtained from the same strain of schistosome

using *Australorbis glabratus* of tropical American stock, 8-8½ weeks after exposure to miracidia. A monkey commenced to pass ova four months after exposure to these cercariae.

2. *Bulinus truncatus*, laboratory-bred from Egyptian stock, proved completely non-attractive to miracidia from a West African strain of *S. haematobium*.

3. The significance of the above facts in relation to the question of host-parasite relationship and distribution is discussed.

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# FIELD TRIALS WITH 'GAMMEXANE' AS A MEANS OF MALARIA CONTROL BY ADULT MOSQUITO DESTRUCTION IN SIERRA LEONE\*

## I.—THE EFFECT OF 'GAMMEXANE' ON MOSQUITOES

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### INTRODUCTION

A continued reduction in the length of life of mosquitoes to below the time which they normally take to become infected would eliminate the possibility of malaria transmission. It has been with this aim in view that residual insecticides have been used to a great extent in recent years. Most of the work has been confined to DDT, which was developed more or less as the direct result of the urgency of war-time conditions. It was not until 1942 that the insecticidal properties of hexachlorocyclohexane (benzene hexachloride,  $C_6H_6Cl_6$ ) were first discovered by Imperial Chemical Industries Limited, and in 1943 the active insecticidal constituent, the gamma isomer, 'Gammexane,' was isolated. This was present to the extent of 13 per cent. of the crude product, and was found to be toxic to the majority of the common insect pests, to many of which it was more toxic than any other insecticide known (Slade, 1945).

There is very little published information on the toxicity of 'Gammexane' to adult mosquitoes, but what little is available indicates not only that 'Gammexane' is lethal to mosquitoes in smaller concentrations than DDT, but that it is quicker in its action. Thus, boards treated with 13 mgm. of 'Gammexane' per sq. ft. gave 100 per cent. knock-down of *A. quadrimaculatus* (confined under Petri-dish covers) in 14–20 minutes, as compared with 80 minutes for DDT, and there was no sign of any decrease in effectiveness eight weeks afterwards.† Other workers using emulsions of benzene hexachloride and DDT found that benzene hexachloride at 13 mgm. per sq. ft. (1.7 mgm. of 'Gammexane') on a glass surface produced 100 per cent. mortality of *Culex fatigans* up to four days after treatment, with a contact-time of only five minutes, and that DDT at 22 mgm. per sq. ft. produced a mortality of only 35 per cent., with a contact-time of 60 minutes. Hence it was concluded that the crude benzene hexachloride was at least 24 times as active as DDT immediately after treatment. At the end of 17 days after treatment the benzene hexachloride was still six times as active as DDT. Also, using solutions of the insecticide in toluene, it was found that benzene hexachloride at only 6.1 mgm. per sq. ft. (0.8 mgm. of 'Gammexane') produced a mortality of 100 per cent. in the first four days after treatment, with a contact-time of five minutes, and that 17

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days after treatment the glass surface treated with benzene hexachloride required only half the time needed by DDT to produce 50 per cent. mortality at 32 mgm. per sq. ft. (30 : 80 minutes). In this case the benzene hexachloride was at least 50 times as active as DDT in the initial stages, and about 12 times as active after 17 days. Observations made during these experiments indicated a consistently more rapid knock-down with benzene hexachloride, and it was noticed that insects knocked down by benzene hexachloride never recovered, while a fair number of those knocked down by DDT showed signs of recovery (Cawnpore Ordnance Laboratories, 1945a).

The object of the present work was to measure the true value of 'Gammexane,' applied as a residual insecticide, as a means of malaria control by adult mosquito destruction in Sierra Leone. It was intended to use the insecticide in areas where *A. gambiae*, *A. funestus* and *A. melas* were the prevalent malaria-carrying mosquitoes, and it was thought that the thorough treatment of an area where *A. melas* was the main mosquito might eradicate this species, as the breeding of it is more or less limited to isolated zones of mangrove (*Avicennia*) swamp on the coast (Thomson, 1945).

Trials with the insecticide were carried out entirely on a practical scale, using ordinary native villages and the better-constructed quarters erected by an iron-ore mining company for the housing of labourers. The intention was to begin control before the wet season commenced and to carry it on to the end of that season. The use in the houses of smoke-generators containing 'Gammexane' was thought to be the ideal and simplest method of effecting this control, and these were used at first. Under the existing conditions of housing, however, they did not prove very successful in reducing the mosquito density, and they were later replaced by residual sprays containing the insecticide, but only when the wet season had already begun.

Work commenced in April, 1946, on several villages on the coast of the Colony of Sierra Leone, south-west of Freetown. In June, 1946, it was decided to make similar trials inland, in the villages and labourers' quarters surrounding the iron-ore mine of the Sierra Leone Development Company at Marampa, 80 miles east of Freetown in the Protectorate of Sierra Leone, and in September, 1946, the large coastal village of Wellington, six miles east of Freetown, was used for trials with different forms and concentrations of 'Gammexane.'

## GENERAL METHOD OF EXPERIMENTS

### *Treated and Control Villages*

The original intention was to use two villages, within a few miles of each other and similar in mosquito and malaria incidences, and to treat all the houses in one of the villages with 'Gammexane' while leaving the other untreated. It was then proposed to carry out periodic mosquito-catches in both the villages, and to determine, by comparison, for how long a certain type of treatment was effective. Any effect of treatment on the sporozoite-rate of the mosquitoes and on the malaria-rate in the children was also to be investigated.

Several difficulties were encountered, however, which led to a slight modification of this procedure. In the first place it was not possible to find in the same area two suitable villages of sufficient size and population, and several small villages, south of Freetown and at Marampa, were therefore used, treated and control villages being selected

from them at random. In the case of the treated villages it was never possible to treat every room in every house, and in each village a note was made of houses treated and of those untreated. Maps were made of the villages for this purpose, and the houses were numbered.

In the case of Wellington, a comparatively large village, portions were treated with different types and concentrations of 'Gammexane,' and individual houses, scattered among and between the treated areas, were used as controls.

### *'Flit-Spraying' Method of Estimating Mosquito Densities*

To ascertain the numbers of adult mosquitoes in houses the method of 'flit-spraying' with sheets was used. White sheets, approximately 8 ft. by 6 ft., were spread over the whole of the floor of the room to be sprayed, as well as over the furniture and, where necessary, over and under the beds. All doors and windows were then closed, and the room was thoroughly sprayed with pyrethrum-kerosene solutions by means of continuous-pressure flit-guns. The pyrethrum-kerosene solutions were made up from Stafford-Allen Pyefly extract (pyrethrins content 6.5 per cent.): in the Freetown area, 0.1 per cent. pyrethrins in kerosene; in the Marampa area, 0.08 per cent. pyrethrins in a kerosene-petrol mixture (64 pints kerosene to 15 pints petrol). The addition of petrol improved atomization. Both mixtures had good knock-down properties and the sheets were removed 5-10 minutes after spraying, the mosquitoes being collected in wide glass specimer-tubes containing slightly damped cotton wool. This method of transportation enabled identification and dissection of the majority of the specimens to be carried out six or seven hours later.

As far as possible a representative sample of houses from each of the villages, both treated and control, were sprayed each week, the usual number being five houses, two rooms in each house. Our aim was that, guided by the maps and house-numbers, different houses would be sprayed on succeeding weeks, so that in a village of 30 houses each individual house would be sprayed only once every six weeks. This was an attempt to eliminate any control by actual 'flit-spraying.'

### *Tabulation of Results of 'Flit-Spraying'*

In the tables and histograms\* included in this report, the flit-catches for each area are grouped weekly or monthly, and room-indices are calculated by dividing the total number of mosquitoes and the number of anopheline vectors by the number of rooms examined in that week or month. Some of these figures have been graphically recorded in columns, the whole column representing the total number of mosquitoes per room per week, and the shaded portion of the column representing the number of anopheline vectors per room per week. The week in all cases is from Sunday to the following Saturday.

In the case of treated areas arrows are inserted on the histogram to show where treatment was carried out.

Untreated houses within treated villages were often included in the 'flit-catching' programme, but in most cases, the number of such houses being small, no attempt has

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\* In order to economize space, the tables on which the histograms are founded have been omitted. These are in the possession of the author and can be supplied to those interested.

been made to separate them in the final presentation of the results. This point is dealt with more fully at a later stage.

In the case of the 'smoke' treatment of Sussex and the 'boys' quarters and in all the treatments of Wellington (where no control village was used), treated and untreated houses in the same area have been separated.

#### *Treatment of Villages with 'Gammexane'*

Treatments were of various types and will be described in more detail later. Small smoke-generators containing 'Gammexane,' and various concentrations and types of 'Gammexane' in the form of residual sprays, were used.

#### *Mosquito Dissections*

Small numbers of dissections were carried out on mosquitoes collected from many of the villages in August and September, 1946, to determine whether treatment with 'Gammexane' would lower the sporozoite-rate.

### DESCRIPTION OF AREAS USED FOR THE EXPERIMENTS

#### VILLAGES ON THE SOUTH-WEST COAST OF THE COLONY OF SIERRA LEONE (Map 1)

Seven villages on the coast of the Colony of Sierra Leone, south of Freetown, were eventually incorporated in the investigations, four being treated with 'Gammexane' and three being used as controls. Table I gives a list of these villages, their populations and tribes and the numbers of inhabited houses. In addition, an isolated house, a Public Works Department compound, situated about half-way between Hamilton and Sussex and about half a mile inland, served as another control (Plate VIII, fig. 6).

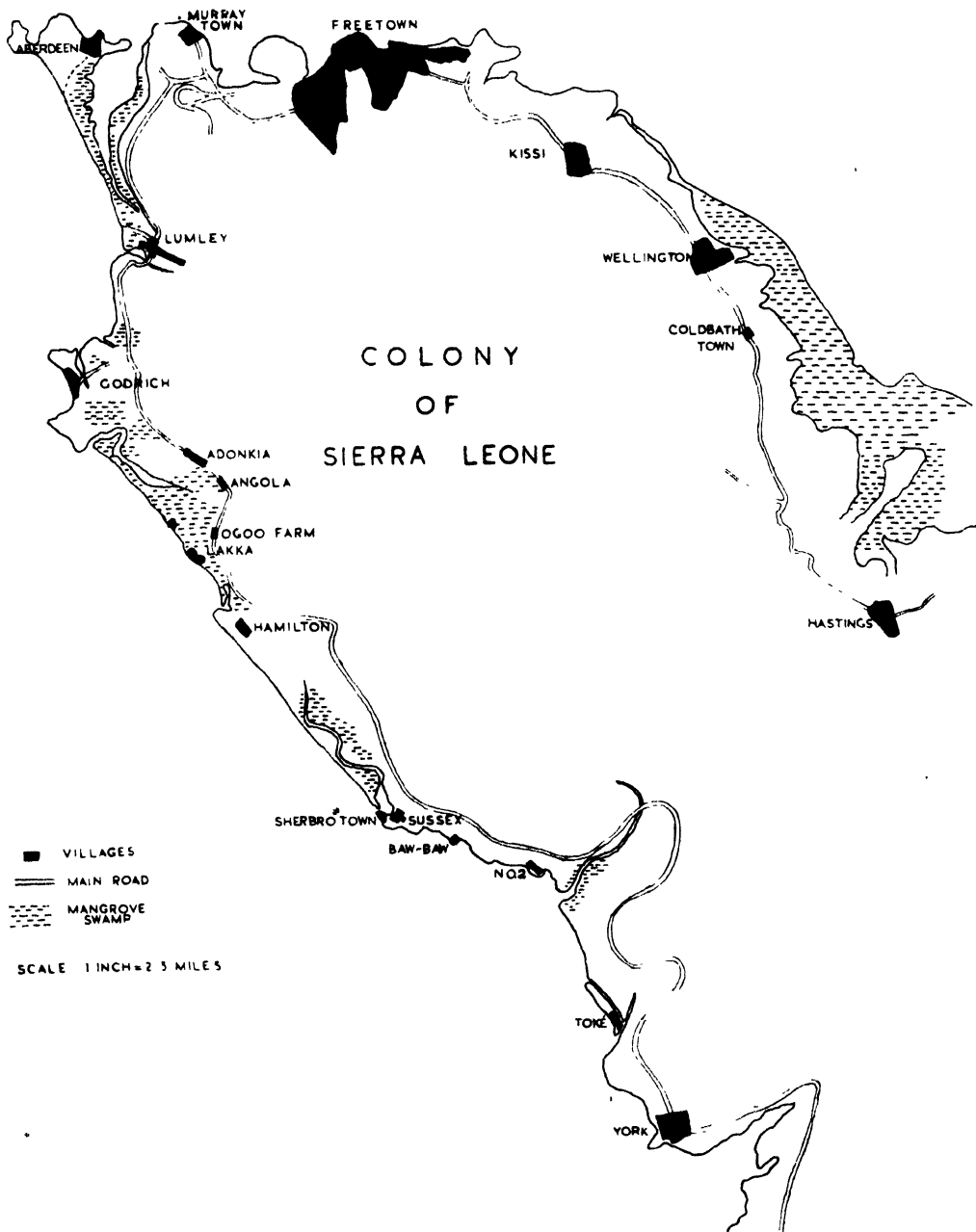
TABLE I  
Villages on the south-west coast of the Colony of Sierra Leone

Village	Tribe	Population, 1944	No. of inhabited houses
Ogoo Farm ...	Temne	144	18
Lakka ...	Sherbro	158	27
Hamilton ...	Creole	203	43
Sussex ...	"	140	39
Sherbro Town ...	Sherbro	198	43
Baw-Baw ...	"	304	54
No. 2 River ..	"	118	31

All these villages lie due south of Freetown and, except for Ogoo Farm, which lies half a mile inland, all are coastal.

Ogoo Farm is 10 miles from Freetown by road, six miles as the crow flies. Lakka lies half a mile south-west of Ogoo Farm. Other distances are: Lakka to Hamilton, 1 mile; Hamilton to Sussex and Sherbro Town, 3 miles; Sussex to Baw-Baw, 1 mile; and Baw-Baw to No. 2 River,  $1\frac{1}{2}$  miles.

Sussex and Sherbro Town, although two distinct villages inhabited by different tribes, are situated very close to one another, and both were treated with 'Gammexane,' along with Lakka and No. 2 River.



MAP 1. The Colony of Sierra Leone.

Small areas of mangrove swamp occur along this coast, associated with river-mouths. As far as could be ascertained most of this mangrove is *Rhizophora*, though some *Avicennia* does occur; one small island of *Avicennia* was located between Hamilton and Sussex, but no actual breeding of *A. melas* was found there. *A. melas* adults, however, were fairly numerous in most of the villages (Table IX).

### *Types of Houses*

The Sherbro and Temne villages (Lakka, Sherbro Town, Baw-Baw, No. 2 River and Ogoo Farm) are mostly composed of square houses with mud walls and sloping thatched roofs (Plate VIII, fig. 6). The Creole villages (Sussex and Hamilton) show a variety of houses (Plate VII, figs. 1-4). Table II gives an analysis of the types of houses in three of the villages.

TABLE II  
Types of houses in three of the coastal villages

Village	No. of houses examined	Mud walls, thatched roof	Wood walls, thatched roof	Wood walls, corrugated-iron roof	Mud walls, corrugated-iron roof
Sussex ...	39	17	9	13	0
Sherbro Town	39	27	3	4	5
Lakka ...	27	20	0	0	7

In most of the houses there is a gap varying from six inches to two feet between the top of the wall and the roof. Each house is divided into two, three or four bedrooms, a 'parlour' and sometimes a kitchen, although often the kitchen is in a separate small outhouse. A few two-storied houses occur in the Creole villages (Plate VII, figs. 2-3).

### *Species of Mosquitoes* (Table IX)

The majority of the mosquitoes found were anopheline vectors, viz., *A. gambiae* and *A. melas*; no other vectors were found, the only other species of anopheline identified being *A. rhodesiensis*, and this only in very small numbers. Small numbers of culicines were recorded but not identified.

*A. melas* was separated purely on the adult character, the four-banded palp (Ribbands, 1944a.) (A more certain differentiation would, of course, have been founded on the egg pattern, but such examination was not a practical possibility.) This species occurred as a fairly large percentage of the total mosquitoes in Sussex, Sherbro Town, Hamilton and Baw-Baw, but only as a small percentage in the other villages.

### *Mosquito Density* (Fig. 1)

In all the villages in this part of the Colony a marked peak in the mosquito density occurred in June, beginning towards the end of May and decreasing to almost nil by the end of July. This peak occurred after the 'small rains' at the beginning of the wet season, but during the wettest part of the year, i.e., July to September, the numbers of mosquitoes were few.

The short-lived peak in the mosquito density in this area indicates that the temporary breeding-places formed during the early rains are later washed out by the heavy rains and that breeding is thus prevented. In this connection the scarcity of permanent collections of fresh water suitable for mosquito breeding was very noticeable.

### MARAMPA AND DISTRICT (Map 2)

The iron-ore mine of the Sierra Leone Development Company is situated about 80 miles east of Freetown in the Protectorate of Sierra Leone, and is surrounded by several native villages and special quarters built by the Company for the housing of labour. Ten native villages were used for the investigations, a list of which is given in Table III, together with the numbers of occupied houses in each.

TABLE III  
Villages in the Marampa area

Village	No. of occupied houses
Magbenkiti ... ..	26
Mafawki ... ..	26
Rochendata ... ..	30
Moria ... ..	6
Katik ... ..	24
Rogbaneh* ... ..	20
Lunsar* ... ..	150-200
Rokontah* ... ..	40
Batabana ... ..	6
Magbil ... ..	12

\* Control villages.

In all these villages, except Lunsar, the predominant type of house is the round-wall, conical-roof native type, with mud walls and a thatched roof (Plate VIII, fig. 7). The main part of the house is the large central kitchen-parlour, around which are arranged the bedrooms, which often number as many as 10. A few of the houses in some of these villages and the majority of the houses in Lunsar are square or rectangular in plan, with mud walls and a sloping thatched or corrugated-iron roof (Plate VIII, fig. 8).

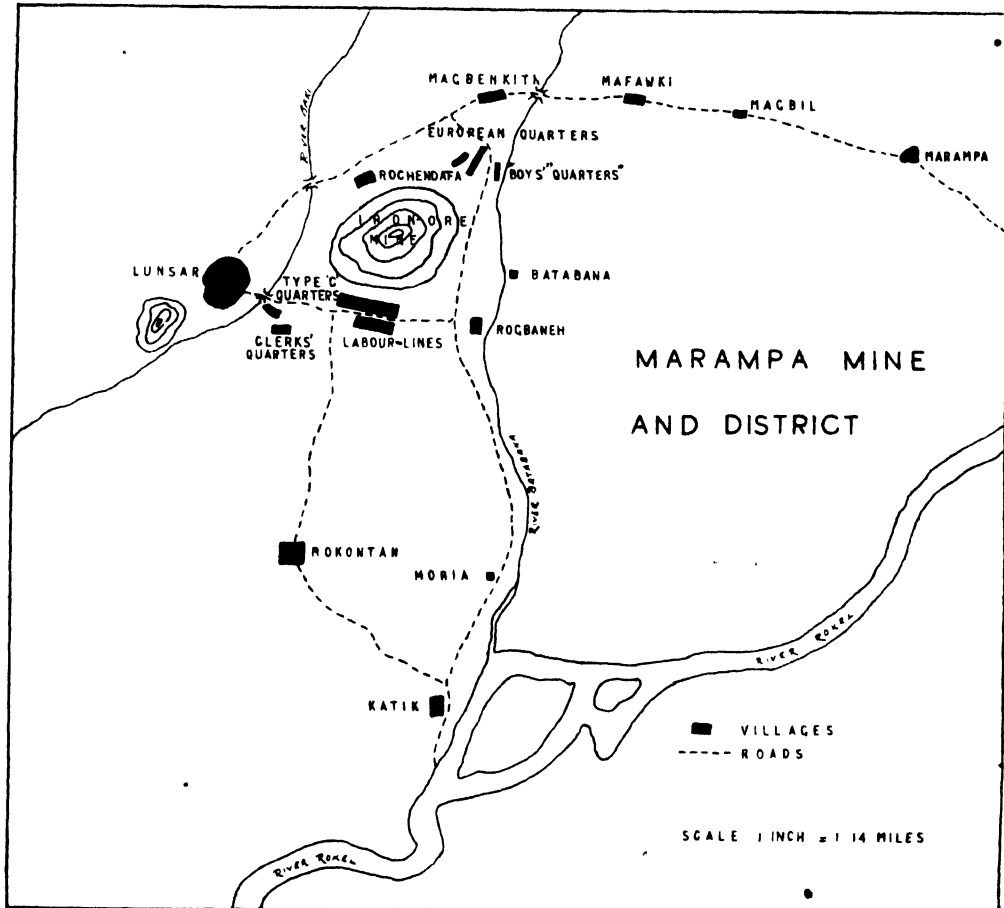
The native-labour quarters are in four sections, as shown in Table IV.

TABLE IV  
Labourers' quarters in the Marampa area

	No. of blocks	No. of rooms in each block
'Boys' quarters ... ..	9	5
Clerks' " ... ..	8	6
Type 'C' " ... ..	6	7
Concrete blocks of the labour-lines ... ..	16	6

Labour-lines—mud houses : 116 houses, 4 rooms in each

The 'boys' quarters, clerks' quarters and type 'C' quarters (Plate IX, figs. 9, 10, 11) are all composed of blocks of rooms in a row, with whitewashed concrete walls and a sloping corrugated-iron roof. These are all of the same size in the 'boys' quarters and clerks' quarters, but in the case of the type 'C' quarters, where there are seven rooms in each block, the middle one is larger than the others and serves as a 'parlour.' The labour-lines are composed both of mud-walled thatched-roof houses (Plate IX, fig. 12) with four rooms in each, arranged in a square, and of concrete blocks of rooms similar to those of the clerks' quarters.



MAP 2. The Marampa iron-ore mine and district in the Protectorate of Sierra Leone.

The European staff of the mine are housed in about 20 bungalows, each with adjacent outhouses, situated on two ridges on the north side of the mine. They have concrete walls and corrugated-iron roofs, and are all screened with wire mosquito-netting.

Lunsar, Rochendata, Magbenkiti, Mafawki, 'boys' quarters, Batabana, Rogbaneh, labour-lines, clerks' quarters and type 'C' quarters all lie more or less in a circle around



the mine, all within one mile of it and of each other. Magbil lies one mile east of Mafawki, Rokontah and Moria lie two miles south of the mine, and Katik about three miles south on the edge of the river Rokel.

Numerous large and small collections of water, both natural and artificial, many of them permanent, occur throughout the area.

#### *Species of Mosquitoes* (Table IX)

*A. gambiae* was by far the commonest species of mosquito found in the area. *A. funestus* was present in moderate numbers in Rochendata but only in small numbers elsewhere. One female of each of *A. nili* and *A. hancocki* were the only other vectors recorded. Other anopheline species recorded very occasionally were *A. rhodesiensis* and *A. coustani* var. *ziemanni*. Culicines were few in number.

#### *Mosquito Density*

Records for 1945 and part of 1946 (Table XI) show a moderate mosquito density over the whole year, with the greatest density in the first seven or eight months of the year. Densities in 1945 were considerably higher than those in 1946 (fig. 2). There does not seem to be any obvious relation between rainfall and mosquito density. The heaviest rainfall occurs from July to October, as in other parts of Sierra Leone (fig. 2).

The more uniform distribution over the year of the mosquito densities in the Marampa area, as compared with those of the coastal villages south of Freetown, is associated with the occurrence of large permanent collections of water, which make the influence of the rainfall less marked, although the heavy rains do appear to cause some reduction.

#### WELLINGTON (Map 1)

Wellington is a large Creole village of some 200 houses, situated five miles south-east of Freetown, on the coast of the Colony of Sierra Leone. The houses are of various types, as in Sussex and Hamilton (Plate VIII, fig. 5). Investigations were confined to that part of the main village which lies on the coastal side of the main Freetown-Hastings road, and to a more or less isolated part, designated Wellington-East, at the eastern end of the village and on the inland side of the road. The coastal part is adjacent to an area which was previously mangrove swamp and has now been 'bunded' and cleared (Thomson, 1945). Many pools in the laterite rock and overgrown ditches occur within the village itself, and mosquito-breeding was found in some of them. Wellington-East is surrounded by a flat grassy area with much surface-water and many ditches.

A few observations were made in Coldbath Town, a small village about half a mile south-east of Wellington.

#### *Species of Mosquitoes* (Table IX)

Again the majority of the mosquitoes were anopheline vectors. *A. gambiae* was the commonest species, but *A. funestus* was also present in large numbers. *A. melas* was also found, but only in small numbers. One female of each of *A. nili* and *A. hancocki* were the only other vectors of importance to be recorded. The only other species of *Anopheles* identified was *A. rhodesiensis*. Small numbers of culicines occurred.

### *Mosquito Density*

Examination of the figures for 1944 and 1945 (Table X) shows a sharp rise in the mosquito density in June, which decreases gradually towards the end of the year.

### TREATMENT OF HOUSES WITH SMOKE-GENERATORS CONTAINING 'GAMMEXANE'

The no. 2 smoke-generator, as supplied by Imperial Chemical Industries Limited, is a 2 oz. pellet containing 25 per cent. benzene hexachloride, of which 13 per cent. is the gamma isomer, i.e., the 'Gammexane' content is approximately 1.8 gm., of which up to 50 per cent. may be destroyed by the heat generated in the burning of the pellet.

Preliminary trials with these smoke-generators in sealed rooms have indicated a persistent effect for from four to eight weeks. In a room of approximately 800 c. ft. in the Ross Institute, London, one generator produced a persistent effect for at least four weeks (private communication from Professor G. Macdonald, Director of the Ross Institute). In Assam, two generators were set off in a room of 2,420 c. ft., and eight weeks later house-flies and anopheline and culicine mosquitoes liberated into the room were killed (private communication from Dr. G. C. Ramsay, Ross Institute, India).

In the areas in Sierra Leone treated with these smoke-generators, one pellet was used per room where possible, the rooms varying from 500 to 2,000 c. ft. Houses were closed as efficiently as possible before 'smoking' and were left closed for one hour afterwards.

The areas treated in this manner were Sussex and Sherbro Town, the 'boys' quarters at Marampa and Wellington-East. In all cases there was much leakage of the smoke, mainly through the open eaves, as well as through the thatch roof, where present, and through badly-fitting doors and windows. In many cases the smoke had completely cleared from the houses in 15-30 minutes.

Table V shows the dates of treatment and the percentage of houses treated in each area.

TABLE V  
Treatment of four villages with 'Gammexane' smoke-generators

Date	Village	Percentage of houses treated	No. of pellets	No. of pellets per house
7.5.46	Sussex	79	98	2-5
15.5.46	"	72	83	1-4
28-29.5.46	"	74	82	1-5
8.5.46	Sherbro Town	91	106	2-4
20.6.46	'Boys' quarters	53*	24	1†
4.7.46	"	44*	20	1†
12.9.46	Wellington-East	81	36	1-6

\* Percentage of rooms treated. Rooms not treated on 20.6.46 were done on 4.7.46.

† One pellet per room.

### RESULTS OF SMOKE TREATMENTS

#### (i) *Sussex*

Sussex was first 'smoked' when the mosquito density was very low, viz., two mosquitoes per room, prior to the June peak. For the first week after treatment the

number of mosquitoes per room in the treated houses was about half that in the untreated houses, but the same as that in the control villages. The village was then treated again, and for the first week after this second treatment the mosquito density was very much lower in treated houses than in untreated ones, and considerably lower than in the control villages. After two weeks the density was a little higher in the treated houses than in the untreated ones and very much higher than in the control villages. The first week after a third smoking the density in the treated houses was still high and approaching that of the control villages.

Table VI summarizes these results in periods of weeks after treatment.

TABLE VI  
Results of treatment of Sussex with smoke-generators containing 'Gammexane.'  
Weekly mosquito room-indices

Weeks after smoking	Treated houses		Untreated houses		Control villages	
	Total mosquitoes	Vectors	Total mosquitoes	Vectors	Total mosquitoes	Vectors
1st 'smoking'	7.5					
1st	46	2.25	1.8	4.1	3.7	2.2
2nd 'smoking'	15.5					
1st	46	2.65	2.25	20.0	19.0	7.7
2nd		36.4	31.0	29.0	24.0	8.5
3rd 'smoking'	28-29.5					
1st	46	18.3	12.6	No observation	30.3	21.9

An interesting individual result was obtained from one house: on May 28th it was found to contain 89.5 mosquitoes (77.5 vectors) per room; on the day after 'smoking' the room-index was again high, being 13.5 mosquitoes (8.5 vectors).

To investigate whether mosquitoes from treated houses were in any way affected by the insecticide, about 30 female *A. gambiae* were caught alive from a treated house in Sussex five days after its treatment, and nine female *A. gambiae* from a house in Hamilton, one of the control villages. Both batches were kept in separate cages under identical conditions, and after five days all of them were still alive.

Efforts were also made to recover dead bodies of mosquitoes from treated houses. Sheets were laid on the floor in three rooms in two houses which had been 'smoked' two days previously. They were left overnight, but no dead mosquitoes were recovered the next day.

#### (ii) Sherbro Town (Fig. 1)

At the time when Sherbro Town was 'smoked' the mosquito density was very low—one per room—and after treatment it showed very little sign of rising until a month later, when there was a very slight increase to four mosquitoes per room, coinciding with the June peak in the other villages in the same area. During the observations Sherbro Town never at any time, right up to the end of October, showed a high mosquito density, and it is thought that this village, without treatment, would have remained low in its

mosquito-population, and that therefore no reliable conclusions can be placed on the data obtained there.

(iii) 'Boys' Quarters

On June 20th, 1946, when the number of mosquitoes per room was 18 (17 vectors), it was only possible to 'smoke' about half the rooms in the 'boys' quarters. Two weeks afterwards the mosquito density in these treated rooms was 8.3 mosquitoes (5.7 vectors) per room, as compared with 40.3 mosquitoes (37.3 vectors) per room in the untreated rooms and 33.4 mosquitoes (29.8 vectors) per room in the control villages. The majority of the untreated rooms were then treated, and another two weeks later (four weeks after the first 'smoking') the room-indices were:

All treated rooms	...	...	26.6 (22.6 vectors)
Rooms treated on 20.6.46	...	...	23.8 (19.0 " )
" " 4.7.46	...	...	35.0 (33.5 " )
Control villages	...	...	7.9 ( 7.3 " )

indicating that the insecticide was effective for more than two, and less than four, weeks in the case of the first batch of rooms treated, and for less than two weeks in the case of the second batch.

(iv) Wellington-East

Treatment was carried out in Wellington-East when the mosquito density in the whole of Wellington was high, viz., 35.5 mosquitoes (20.7 vectors) per room. In succeeding weeks the room-indices for treated and control houses were:

TREATED HOUSES	CONTROL HOUSES
1.0 (0.4 vectors)	4.3 (3.4 vectors)
6.1 (4.5 " )	11.1 (8.4 " )
4.3 (2.8 " )	4.8 (3.4 " )
2.6 (1.6 " )	2.1 (1.1 " )

indicating a possible persistence for three weeks, though there was a marked rise in the mosquito density in the treated houses only two weeks after treatment.

#### GENERAL CONCLUSIONS

The foregoing results indicate that, under the conditions of the trials, smoke-generators containing 'Gammexane' are not an efficient means of adult mosquito control. Although some control is effected for 1-3 weeks after treatment, it is not nearly so well marked as in the case of treatment with residual sprays (see below). The fact that one house in Sussex, only 24 hours after smoking, showed a mosquito density of 13.5 per room confirms this inefficiency, as also does the fact that adults from a treated house remained alive for five days, and that no bodies of dead mosquitoes were recovered from treated houses.

The obvious explanation for this inefficiency is the inadequate retention of the smoke by the houses which were used. These houses—and houses generally in the tropics—are impossible to seal sufficiently for the use of 'smokes' without a great amount of preliminary work, which would eliminate their use on a practical scale for mosquito control. Moreover, such measures would cause some inconvenience to the inhabitants, who have to remain out of their houses for at least one hour after treatment.

## TREATMENT OF HOUSES WITH RESIDUAL SPRAYS CONTAINING ' GAMMEXANE '

### *Types of ' Gammexane ' and Concentrations*

For the most part solutions of the gamma isomer of benzene hexachloride (' Gammexane ') in kerosene were used for residual spraying, but later other products were employed. Table VII gives a list of the products used and their ' Gammexane. ' content.

TABLE VII  
Products used for residual sprays and their ' Gammexane ' content

Description	' Gammexane ' content
Gamma isomer of benzene hexachloride ... ..	95 per cent.
Dry crude benzene hexachloride, ground . . . . .	13 "
Water-dispersible powder, 50/50 benzene hexachloride/goulac ... ..	6 "
Water-miscible oil, ' Gammexane ' dissolved in 15 per cent. sextone, 80 per cent. turkey-red oil	5 "

Forty-five gm. (1.6 oz.) per gallon of kerosene was taken as a 1 per cent. solution of the gamma isomer (it is actually 1.18 per cent.). Concentrations of 0.33 per cent., 0.5 per cent. and 1 per cent. were used. Other products and concentrations which were used were: water-miscible oil, mixtures containing 0.35 per cent. and 1 per cent. ' Gammexane ' ; water-dispersible powder, mixtures containing 0.35 per cent. and 0.5 per cent. ' Gammexane ' ; and crude benzene hexachloride, solutions in kerosene containing 0.1 per cent., 0.2 per cent. and 0.4 per cent. ' Gammexane ' approximately.

The water-miscible oil and water-dispersible powder had the advantage of only requiring water for dilution and could be made up ' on the spot. ' The water-miscible oil was immediately miscible, and remained so. The water-dispersible powder mixture was made up in an open drum or bucket, first by adding just enough water to make a cream, and then by adding the remaining bulk of water and stirring. There was a tendency for the powder to sink to the bottom of the container, and frequent stirring during application was therefore necessary.

Both the gamma isomer and the crude benzene hexachloride were slow to dissolve in kerosene at normal temperatures, and solutions were consequently left, with frequent stirrings, for usually at least one week before use. A 3 per cent. solution of the benzene hexachloride in kerosene was saturated.

### *Machines Used for Residual Spraying*

For the spraying of kerosene solutions and water mixtures containing the water-miscible oil, Kent pneumatic sprayers, of 1 gallon capacity, were used. For the spraying of water-dispersible powder mixtures, stirrup-pumps fitted with the Kent pneumatic sprayer-arm and nozzle were used. In one case, at Wellington, the water-dispersible powder mixture was applied with a Four Oaks portable lime-washing machine.

Application-rates and efficiencies of two Kent sprayers\* and one stirrup-pump were

\* The average of figures obtained at 25, 50 and 75 lb. pressure was taken, as both the machines leaked to some extent and the recommended pressure of 75 lb. per sq. in. was not always maintained.

worked out in the laboratory by spraying solutions on to sheets of a known area and recording the increase in weight, and by estimating the delivery-rate over a period of one minute. The results of these experiments are given in Table VIII. The average adherence-rate for the two Kent sprayers was 3.99 pints per 1,000 sq. ft., and the average efficiency (i.e., percentage of spray delivered which actually adhered to the sheet) was 76.6 per cent. For the stirrup-pump fitted with the Kent nozzle these figures were 7.74 pints per 1,000 sq. ft. and 88.2 per cent.

TABLE VIII  
Calibration data of the machines used for residual spraying

	Pressure, lb. per sq. in.	Delivery, pints per minute	Adherence, pints per 1,000 sq. ft.	Efficiency, percentage
Kent pneumatic sprayer I	25	0.76	4.00	86.8
	50	1.05	4.96	71.4
	50	1.05	4.78	89.5
	75	1.25	4.50	68.8
	75	1.25	4.08	75.2
Average adherence : 4.46 pints per 1,000 sq. ft. " efficiency : 78.3 per cent.				
Kent pneumatic sprayer II	25	0.85	3.37	84.7
	25	0.85	3.19	63.5
	50	1.05	3.27	71.4
	50	1.05	4.07	89.5
	75	1.22	3.09	68.0
	75	1.22	4.05	70.5
Average adherence : 3.51 pints per 1,000 sq. ft. " efficiency : 74.9 per cent				
Stirrup-pump with Kent sprayer-arm and nozzle attached			7.02	86.7
			6.48	95.6
		Average 0.90	8.78	71.1
			10.16	96.7
			8.11	95.6
			5.88	83.3
Average adherence : 7.74 pints per 1,000 sq. ft. " efficiency : 88.2 per cent.				

### *Technique of Spraying*

The nozzle of the spraying machine was held approximately one foot away from the wall surface to be sprayed, when the cone diameter of the spray was approximately 15 inches. All side-walls and as much of the roof as could be reached were sprayed. Application-rates in mgm. of 'Gammexane' per sq. ft. have been calculated from the calibration data of the machines used, viz., 4 pints per 1,000 sq. ft. in the case of the Kent sprayer and 7.74 pints in the case of the stirrup-pump. Also, in many of the villages rough estimates were made of the area sprayed and of the volume of spray used, and the application-rate, assuming an efficiency of 75 per cent. in the case of the Kent sprayer and of 88 per cent. in the case of the stirrup-pump, was calculated. The

application-rate, where calculated, is always considerably lower than that calculated from the machine calibration data, owing to the fact that the calculated efficiency of the machines is probably an underestimate for the surfaces treated, which would be more absorbent than the sheets used in the actual calculation of the efficiency of the machines. Moreover, estimates of areas sprayed were very approximate. The true application-rate can be said to occur between the two extremes.

### *Types of Wall Surface*

In the majority of houses it was only possible to spray the side-walls of the rooms, and note was made in each case of the type of wall surface. All the treated villages south of Freetown, except Sussex, consisted of houses with, for the most part, mud walls. In the case of Sussex 40 per cent. of the houses had wood walls. All the labour quarters at Marampa, except the mud houses of the labour-lines, had walls of whitewashed concrete; all the treated villages had mud walls. The houses in Wellington showed a large variety of wall surface: wood, mud, thatch, concrete and corrugated iron.

TABLE IX

Analysis of the species of mosquitoes recorded from the areas under observation in Sierra Leone

Village	<i>A. gambiae</i> group				<i>A. funestus</i>		Culicines
	Female <i>A. gambiae</i> type	Female <i>A. melas</i>	Unidenti- fiable females	Males	Female	Male	
COASTAL VILLAGES SOUTH OF FREETOWN							
Ogoo Farm ...	1,134	19	63	247	0	0	89
Lakka ...	336	17	65	99	0	0	20
Hamilton ...	518	103	53	93	0	0	214
Sussex ...	479	118	92	72	0	0	102
Sherbro Town ...	41	16	9	4	0	0	14
Baw-Baw ...	196	36	301	17	0	0	11
No. 2 River ...	450	11	860	88	0	0	23
P.W.D. compound	160	1	12	20	0	0	1
MARAMPA AND DISTRICT							
Lunsar ...	947	0	0	69	27	2	40
Labour-lines ...	724	0	0	45	30	2	28
Rochendata ...	927	0	0	146	273	13	37
'Boys' quarters ...	421	0	0	59	9	2	10
Mafawki ...	961	0	0	178	24	1	6
Magbenkiti ...	370	0	0	59	2	0	14
Magbil ...	237	0	0	18	3	1	4
Type 'C' quarters	148	0	0	4	1	0	15
Clerks' "	43	0	0	13	2	0	3
Rogbaneh ...	498	0	0	28	0	0	6
Batabana ...	35	0	0	3	0	0	1
Moria ...	75	0	0	13	1	0	0
Katik ...	156	0	0	14	0	1	0
Rokontah ...	179	0	0	20	3	0	3
WELLINGTON AND DISTRICT							
Wellington ...	973	76	49	405	416	77	64
Wellington-East ...	142	28	0	66	100	36	24
Coldbath Town ...	10	12	4	3	1	1	2

TABLE X  
Monthly mosquito room-indices\* (anopheline vectors) for the villages of  
Aberdeen and Wellington in the years 1941, 1942, 1944 and 1945

Month	Aberdeen		Wellington	
	1941	1942	1944	1945
Jan. ...	4.6	1.1	2.2	1.2
Feb. ...	2.6	0.7	3.7	2.2
March ...	1.1	0.2	0.9	0.9
April ...	0.4	1.4	1.7	0.9
May ...	0.7	0.6	10.7	1.4
June ...	20.0	17.0	22.3	9.0
July ...	16.8	20.0	10.5	8.3
Aug. ...	14.0	12.4	19.8	4.0
Sept. ...	11.4	20.4	12.8	3.8
Oct. ...	1.1	15.2	23.5	1.9
Nov. ...	0.3	0.9	11.6	1.8
Dec. ...	0.7	1.2	9.5	4.3

\* These figures are based on examination of an average of eight rooms weekly in each village.

TABLE XI  
Monthly mosquito room-indices\* in six areas in the Marampa district for 18 months prior to  
treatment with 'Gammexane'

Month	'Boys' quarters	Labour-lines	European quarters (kitchen store-rooms)	Rochendata	Magbenkiti	Mafawki
1945						
Jan.	(5) 12.8	(7) 20.0	(17) 5.8	(5) 48.0	(4) 16.25	(5) 23.0
Feb.	(5) 7.4	(10) 20.2	(17) 7.8	(6) 54.5	(5) 20.4	(7) 9.9
March	(6) 46.2	(9) 16.8	(18) 26.8	(12) 35.9	(10) 25.2	(8) 38.25
April	(8) 34.1	(13) 11.5	(22) 17.4	(14) 28.6	(15) 21.5	(8) 10.75
May	(8) 28.0	(12) 11.2	(20) 14.85	(10) 25.2	(10) 22.5	(12) 13.6
June	(8) 17.9	(12) 9.3	(20) 12.95	(12) 18.25	(12) 27.6	(9) 14.3
July	(19) 32.6	(12) 11.75	(26) 19.9	(16) 17.6	(13) 25.9	(13) 19.7
Aug.	(24) 10.0	(17) 6.5	(20) 12.3	(24) 8.8	(14) 7.4	(17) 9.6
Sept.	(24) 5.5	(14) 5.7	(22) 7.0	(15) 6.7	(12) 9.1	(10) 6.7
Oct.	(26) 3.3	(15) 7.2	(24) 5.4	(13) 6.5	(12) 6.2	(12) 7.9
Nov.	(24) 4.7	(13) 7.2	(20) 6.0	(18) 5.1	(16) 6.3	(12) 7.0
Dec.	(22) 4.8	(21) 5.1	(19) 7.2	(17) 6.7	(12) 7.1	(13) 7.2
1946						
Jan.	(15) 4.4	(16) 13.25	(15) 6.3	(16) 7.7	(16) 6.7	(9) 6.9
Feb.	(12) 5.3	(16) 7.1	(17) 6.8	(12) 8.2	(12) 9.3	(8) 5.75
March	(21) 4.0	(21) 8.1	(21) 4.9	(14) 7.5	(19) 7.8	(12) 8.7
April	(24) 5.7	(19) 10.9	(23) 7.3	(19) 9.6	(14) 11.0	(15) 8.3
May	(20) 5.7	(24) 5.6	(20) 6.3	(26) 9.6	(23) 7.6	(18) 6.9
June		(21) 9.5	(18) 7.1	(25) 11.9		(22) 18.4

\* Total mosquitoes, vectors and non-vectors.

The number of rooms upon which these indices are founded are shown in brackets before each index

## RESULTS OF TREATMENT WITH RESIDUAL SPRAYS

### Villages South of Freetown (Fig. 1)

#### (i) Lakka

<b>First Spraying.</b>	Date of spraying	... ..	16.5.46 (most) and 23.5.46 (4 houses)
	Material used	... ..	0.33 per cent. 'Gammexane' in kerosene
	Percentage of houses sprayed	... ..	84
	" rooms "	... ..	50
	Wall surface	... ..	Mud
	Calculated application-rate of 'Gammexane'	7 mgm. per sq. ft.	



Spraying was carried out when the mosquito density was moderate—5–6 mosquitoes per room—and on the increase. One week after spraying the mosquito density had risen to 6·0 mosquitoes (4·75 vectors) per room, and was little different from the control villages' level of 8·8 mosquitoes (7 vectors) per room. A week later, although considerably less—6·25 mosquitoes (5·5 vectors) per room—than in the control villages—18 mosquitoes (12·5 vectors)—the density was still significant, and it was decided to respray the village with a higher concentration of 'Gammexane.'

<i>Second Spraying.</i>	Date of spraying	...	...	...	30.5.46
	Material used	...	...	...	0·5 per cent. 'Gammexane' in kerosene
	Percentage of houses sprayed	...	...	...	74
	rooms	...	...	...	Not determined
	Calculated application-rate of 'Gammexane'	...	...	...	11 mgm. per sq. ft.

For two weeks after spraying the mosquito density remained strikingly low, at little more than one mosquito per room, as compared with the high level in the control villages of 24–30 mosquitoes (18–23 vectors) per room. During the third week there was an increase in the treated village to 8·8 mosquitoes (7·9 vectors) per room, but at that time the density in the control villages was at its highest—71 mosquitoes (67 vectors) per room. During the fourth week the mosquito density in Lakka was still much lower—3·4 mosquitoes (2·2 vectors) per room—than in the control villages, where, however, the density was now on the decrease—33 mosquitoes (27 vectors) per room. In the fifth week there was a marked rise in the treated village to nine mosquitoes (five vectors) per room, and as there was a marked decrease in the mosquito density of the control villages—in the sixth week this was 18·3 mosquitoes (15·0 vectors)—it was decided to respray.

<i>Third Spraying.</i>	Date of spraying	...	...	...	8.7.46
	Material used	...	...	...	0·5 per cent. 'Gammexane' in kerosene
	Percentage of houses sprayed	...	...	...	85
	rooms	...	...	...	51
	Calculated application-rate of 'Gammexane'	...	...	...	11 mgm. per sq. ft.

For nine weeks after spraying the mosquito density remained very low, at less than one mosquito per room, but in the control villages, too, the density dropped to less than one mosquito per room five weeks after the third spraying. During the 11th week there was a marked rise to 8 mosquitoes (6 vectors) per room, but without a corresponding rise in any of the control villages, where the density was only one mosquito per room. This rise occurred in both treated and untreated houses and would seem to indicate the end of the effect of the insecticide. Fourteen weeks after spraying, however, the mosquito density had again returned to a low level, two mosquitoes per room, only a little higher than in the control villages, where it was one per room.

## (ii) *Sussex*

Date of spraying	...	...	...	...	11 and 14.6.46
Material used	...	...	...	...	0·5 per cent. 'Gammexane' in kerosene
Percentage of houses sprayed	...	...	...	...	90
rooms	...	...	...	...	71
Wall surface	...	...	...	...	60 per cent. wood, 40 per cent. mud
Calculated application-rate of 'Gammexane'	...	...	...	...	11 mgm. per sq. ft.

Spraying was carried out when the mosquito density was high—19 mosquitoes (14 vectors) per room—and resulted in a marked decrease to almost nil immediately after

spraying. A slight rise occurred in the second (2-3 mosquitoes per room) and fourth weeks (3 mosquitoes per room) after spraying, but this was very much lower than in the control villages, the mosquito density of which varied in that period from 18 to 32 mosquitoes per room. Six weeks after spraying the density was still low (1-2 mosquitoes per room), but the control villages now showed only 4-5 mosquitoes per room. After this time both the treated village and the control villages remained very low in their mosquito-populations (2 or less than 2 mosquitoes per room), with the result that the persistence-time of the insecticide could not be estimated.

### (iii) *Sherbro Town*

Date of spraying	...	...	...	...	14 and 27.6.46
Material used	...	...	...	...	0.5 per cent. 'Gammexane' in kerosene
Percentage of houses sprayed	...	...	...	...	84
"    rooms	...	...	...	...	50
Wall surface	...	...	...	...	80 per cent. mud, 20 per cent. wood
Calculated application-rate of 'Gammexane'	...	...	...	...	11 mgm. per sq. ft.

Sherbro Town was sprayed at about the same time as Sussex, which is situated close by. It was considered that, although the mosquito density was very low (4 mosquitoes per room) as compared with the control villages (30 mosquitoes per room), this was not due to the previous treatment of the village, six weeks earlier, with 'Gammexane' smoke-generators. After spraying, the mosquito density dropped to less than one mosquito per room and remained so as long as observations were continued (approximately five months after spraying).

### (iv) *No. 2 River*

Date of spraying	...	...	...	...	25.6.46
Material used	...	...	...	...	0.5 per cent. 'Gammexane' in kerosene
Percentage of houses sprayed	...	...	...	...	84
"    rooms	...	...	...	...	54
Wall surface	...	...	...	...	Mud
Calculated application-rate of 'Gammexane'	...	...	...	...	11 mgm. per sq. ft.

Immediately before spraying the mosquito density was high—36 mosquitoes (29 vectors) per room—but this was a considerable decrease from the density of the previous week, which was 107 mosquitoes (100 vectors) per room, and, as subsequent records show, was the beginning of the decline in the mosquito density throughout the area. The density of 7 mosquitoes per room calculated for the second week after spraying was composed largely of mosquitoes from two untreated houses, and the density of 6 mosquitoes per room for the fourth week after spraying was composed to a great extent of mosquitoes from one untreated house. The densities in the treated houses during these weeks were actually 0.8 mosquitoes (0.2 vectors) and 3.1 mosquitoes (3.1 vectors) per room respectively, as compared with 18.3 mosquitoes (15.0 vectors) and 4.6 mosquitoes (4.1 vectors) in the control villages in the same weeks. After this time the densities in both the treated village and the control villages remained very low (2 or less than 2 mosquitoes per room).

### *General Conclusions*

There is great difficulty in assessing the persistence of the residual sprays used in these coastal villages south of Freetown, owing to the fact that the mosquito density in

the whole area dropped to a very low level at the end of July, very soon after the residual spraying of Sussex, Sherbro Town, No. 2 River and Lakka (third spraying).

The spraying of Lakka with a 0.33 per cent. solution of 'Gammexane' at the beginning of the rise in the mosquito density did not reduce the density very significantly over the two weeks following the spraying.

The respraying of the same village with 0.5 per cent. 'Gammexane' produced a significant reduction in the mosquitoes over five weeks.

The persistence-time of the third spraying of Lakka with 0.5 per cent. 'Gammexane' is difficult to assess, although the marked rise in the mosquito density, 11 weeks after spraying, strongly indicates that the effect of the insecticide ceased somewhere between nine and 11 weeks after spraying.

Comparison of mosquito room-indices obtained in Sussex, Sherbro Town and No. 2 River after spraying with 0.5 per cent. 'Gammexane' with those obtained in the control villages indicates a persistence of at least six weeks, but of how much longer than six weeks cannot be determined.

#### *Marampa and District*

##### (i) 'Boys' Quarters

Date of spraying	...	...	...	...	17.7.46
Material used	...	...	...	...	0.5 per cent. 'Gammexane' in kerosene
Percentage of rooms sprayed	...	...	...	...	80
Wall surface	...	...	...	...	Whitewashed concrete
Calculated application-rate of 'Gammexane'	...	...	...	...	13 mgm. per sq. ft.

A thorough spraying of this area was carried out when the mosquito density was high—27 mosquitoes (23 vectors) per room—and resulted in a very striking decrease to almost nil, and a very low density (for the most part less than one mosquito per room) was maintained for 19 weeks. In the 20th week after treatment the density increased to 2.6 mosquitoes (2.3 vectors) per room and in the 22nd week to 3.25 mosquitoes (2.5 vectors) per room. The latter density was higher than in the control villages, where it was 1.7 mosquitoes (1.6 vectors) per room.

##### (ii) Magbenkiti (Fig. 2)

<i>First Spraying.</i>	Date of spraying	...	...	...	...	20.6.46
	Material used	...	...	...	...	0.5 per cent. 'Gammexane' in kerosene
	Percentage of houses sprayed	...	...	...	...	77
	" rooms	...	...	...	...	40
	Wall surface	...	...	...	...	Mud
	Calculated application-rate of 'Gammexane'	...	...	...	...	11 mgm. per sq. ft.

Spraying was done when the mosquito density was high—37 mosquitoes (30 vectors) per room. Two weeks afterwards the density—3 mosquitoes (2 vectors) per room—was very much lower than that in the control villages—33 mosquitoes (30 vectors) per room. Four weeks after spraying, however, the density had increased considerably to 28 mosquitoes (23 vectors) per room, and was much higher than in the control villages, where it was only 8 mosquitoes (7 vectors) per room. The village was then resprayed.

<i>Second Spraying.</i>	Date of spraying	...	...	...	...	17.7.46
	Percentage of houses sprayed	...	...	...	...	88
	" rooms	...	...	...	...	54

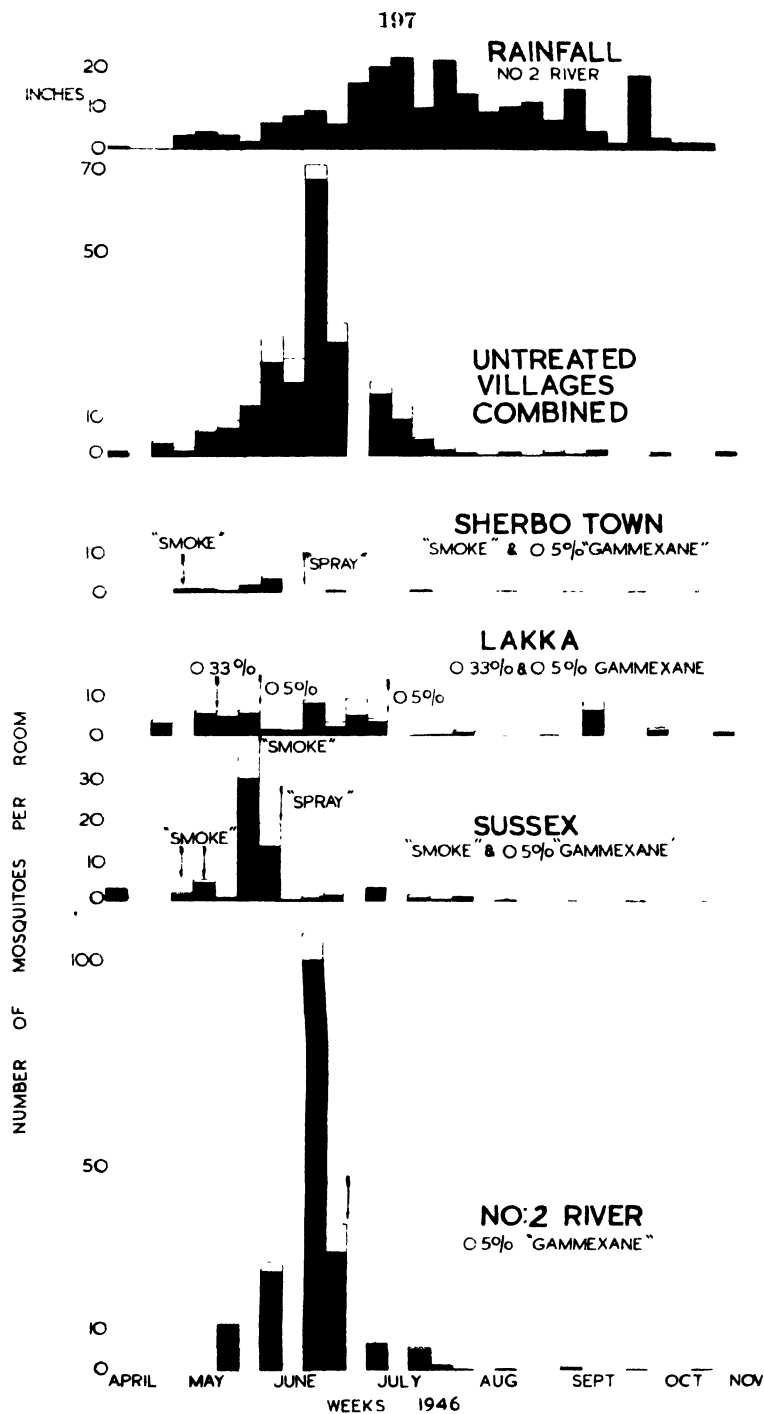


FIG. 1. Rainfall and weekly mosquito room-indices in the coastal villages of Sierra Leone, April-November, 1946.

0.5 per cent. 'Gammexane' in kerosene was again used at the same calculated application-rate and resulted in a marked decrease in the mosquito-population, which remained for the most part below one mosquito per room for a period of 24 weeks. In the three following weeks the density showed signs of rising, and in the 27th week it was 2.25 mosquitoes (2.1 vectors) per room. This, however, was still lower than the density in the control villages, where it was 5.4 mosquitoes (4.5 vectors) per room.

The difference between the persistence-time of the insecticide in these two sprayings is probably largely accounted for by the larger percentage of houses and rooms sprayed on the second occasion.

### (iii) Mafawki

<i>First Spraying.</i>	Date of spraying	3.7.46
	Material used	0.5 per cent. 'Gammexane' in kerosene
	Percentage of houses sprayed	73
	"    rooms	27
	Wall surface	Mud
	Calculated application-rate of 'Gammexane'	11 mgm. per sq. ft.

At the time when Mafawki was first sprayed the mosquito density was very high, being 68 mosquitoes (60 vectors) per room. Two weeks after spraying the density was low—4 mosquitoes (2 vectors) per room—as compared with the control villages, where it was 8 mosquitoes (7 vectors) per room; but three weeks after spraying there was a marked rise to 20 mosquitoes (19 vectors) per room, and this was considerably higher than in the control villages, where it was 7 mosquitoes per room. For the two following weeks, however, the density in the treated village remained a little lower than in the control villages, but in the sixth week it again increased to 15 mosquitoes (13 vectors) per room and was not far below the level in the control villages, where there were 24 mosquitoes (22 vectors) per room. The village was then resprayed.

<i>Second Spraying.</i>	Date of spraying	16.8.46
	Material used	Water-miscible oil mixture containing 1 per cent. 'Gammexane'
	Percentage of houses sprayed	81
	"    rooms	35
	Calculated application-rate of 'Gammexane'	21 mgm. per sq. ft.

For 20 weeks after this second spraying the density remained for the most part below one mosquito per room. In the three following weeks it began to rise, and in the 23rd week after spraying the density was 3.9 mosquitoes (3.4 vectors) per room, as compared with 5.4 mosquitoes (4.5 vectors) per room in the control villages.

The longer persistence of the insecticide on the second spraying can be attributed to the increased application-rate of the 'Gammexane' and to the higher percentage of houses and rooms sprayed.

### (iv) Rochendata

Date of spraying	4.7.46
Material used	0.5 per cent. 'Gammexane' in kerosene
Percentage of houses sprayed	97
"    rooms	44
Wall surface	Mud
Calculated application-rate of 'Gammexane'	11 mgm. per sq. ft.

At the time of spraying the mosquito density was high—44 mosquitoes (40 vectors) per room—and for three weeks afterwards the density remained below that of the control

villages. In the fourth week the number of mosquitoes was about the same in both treated and control villages—6 mosquitoes (5 to 6 vectors) per room—but, instead of respraying, it was decided to observe the mosquito density over subsequent weeks. In the middle of August the mosquitoes in the control villages showed an increase to 24 (22 vectors) per room, whereas the numbers in Rochendata were very much lower—2 (1 vector) per room—and an appreciable difference was maintained until the second and fourth weeks in September, when the density increased to a slightly higher level than in the control villages. From then onwards the densities in the treated villages remained at the same level as, or slightly below, the densities in the control villages. It appears from this that some control was being effected by the 'Gammexane' up till the end of September, 11 weeks after spraying.

(c) *Labour-Lines*

Date of spraying	..	...	...	2 and 3.8.46
Material used	...	...	...	0.5 per cent. 'Gammexane' in kerosene
Percentage of houses sprayed	...	...	...	90 (mud houses)
" rooms	"	"	"	45 (mud houses), 56 (concrete blocks)
Wall surface	...	...	...	Mud and whitewashed concrete
Calculated application-rate of 'Gammexane'	...	...	...	6-11 mgm. per sq. ft.

The spraying of this area produced a marked drop in the mosquito density in the first week, from 7 mosquitoes (7 vectors) per room to 2 mosquitoes (2 vectors) per room,

TABLE XII  
Weekly mosquito room-indices in treated and untreated houses in the labour-lines after spraying with 0.5 per cent. 'Gammexane'

Time of observation	Treated rooms			Untreated rooms		
	No. of rooms examined	No. of mosquitoes per room		No. of rooms examined	No. of mosquitoes per room	
		Total	Vectors		Total	Vectors
Before spraying	...			16	7.5	7.0
Weeks after spraying						
1	14	0.9	0.7	6	3.8	3.8
2	10	2.0	1.6	4	5.5	4.25
3	17	0.5	0.5	3	1.7	1.7
4	17	0.3	0.3	3	7.0	6.3
5	16	1.1	1.0	2	0	0
6	16	1.0	0.4	3	1.3	0.3
7	17	0.4	0.4	5	1.2	1.2
8	16	0.9	0.75	6	0	0
9	15	0.4	0.4	7	0.4	0.4
10	14	0	0	9	1.1	0.9
11	15	0.2	0.1	7	0.3	0.1
12	11	0.2	0.2	9	0.2	0.2
13	14	0.1	0.1	8	0.5	0.4
14	12	0.1	0.1	10	0.7	0.7
15	15	0.5	0.5	7	0.6	0.3
16	14	0.1	0.1	5	0.2	0.2
17	14	0.6	0.3	7	1.1	1.1
18	14	0.9	0.7	6	1.7	1.0
19	15	2.6	2.5	7	2.1	2.1
20	15	1.8	1.6	7	3.0	2.7
21	15	3.7	3.5	7	4.1	3.6

and a low density, for the most part less than one mosquito per room, was maintained for 18 weeks after spraying. In the 19th, 20th and 21st weeks after spraying the density was on the increase and was greater than in the control villages, being in the 21st week 4 mosquitoes (3.5 vectors) per room as compared with the control villages' combined density of 1.4 mosquitoes (1.3 vectors) per room.

(vi) *Clerks' Quarters* (Fig. 2)

Date of spraying	...	...	...	16.8.46
Material used	...	...	...	1 per cent. 'Gammexane' in kerosene
Percentage of rooms sprayed	...	...	...	33
Wall surface	...	...	...	Whitewashed concrete
Calculated application-rate of 'Gammexane'	...	...	...	11-21 mgm. per sq. ft.

At the time of spraying the mosquito density was 12 mosquitoes (9 vectors) per room. For 16 weeks after spraying the density remained well below one mosquito per room. In the 17th, 18th and 19th weeks there was an increase to above the control-village level, this being most marked in the 19th week, when the mosquito density was 8 mosquitoes (7 vectors) per room compared with 1.4 mosquitoes (1.3 vectors) per room in the control villages.

(vii) *Type 'C' Quarters*

Date of spraying	...	...	...	16.8.46
Material used	...	...	...	1 per cent. 'Gammexane' in kerosene
Percentage of rooms sprayed	...	...	...	79
Wall surface	...	...	...	Whitewashed concrete
Calculated application-rate of 'Gammexane'	...	...	...	11-21 mgm. per sq. ft.

Almost exactly the same results were obtained after spraying this area as in the case of the clerks' quarters, although in October the reduction was not so pronounced as in the latter area. This can perhaps be explained by the fact that initially the mosquito density was considerably higher in the type 'C' quarters than in the clerks' quarters, being, immediately before spraying, 31 mosquitoes (30 vectors) per room, indicating that the former quarters were either more attractive to mosquitoes or nearer to the breeding-place. Although only 33 per cent. of the rooms in the clerks' quarters were sprayed, only 50 per cent. of all the rooms in this area were used as bedrooms. In the type 'C' quarters 86 per cent. of all the rooms were bedrooms.

(viii) *Katik*

Date of spraying	...	...	...	2.9.46
Material used	...	...	...	0.5 per cent. 'Gammexane' in kerosene
Percentage of houses sprayed	...	...	...	79
" rooms "	...	...	...	60
Wall surface	...	...	...	Mud
Calculated application-rate of 'Gammexane'	...	...	...	11 mgm. per sq. ft.

Two weeks after spraying there was a marked reduction in the mosquito density, from 31 mosquitoes (29 vectors) per room to 1 mosquito (1 vector) per room. For the following 12 weeks the density remained below one mosquito per room. In the 15th week after spraying the density in Katik rose to a slightly higher level than in the control villages—2.2 (1.4 vectors) per room as compared with 1.8 (1.4 vectors)—but it was not

until after the 18th week after treatment that there was a continued significant increase comparable with the densities in the control villages. In the 20th week the density in Katik was 5.7 mosquitoes (5.3 vectors) per room, and in the control villages 6.3 mosquitoes (5.6 vectors) per room.

(ix) *Magbil* (Fig. 2)

Date of spraying	...	...	...	...	26.9.46
Material used	...	...	...	...	Water-dispersible powder mixture containing 0.35 per cent. 'Gammexane'
Percentage of houses sprayed	...	...	...	...	83
" rooms	"	"	...	...	55
Wall surface	...	...	...	...	Mud
Calculated application-rate of 'Gammexane'	...	...	...	...	8-17 mgm. per sq. ft.

Spraying reduced the mosquito density from 25 mosquitoes (23 vectors) per room to 0.8 mosquitoes (0.3 vectors) per room in the first week. For the four following weeks not a single mosquito was found, and up to eight weeks after spraying the density remained below one mosquito per room. From the ninth to the 14th weeks after treatment the density remained between one and two mosquitoes per room—a slightly lower density than in the control villages, where it varied from one to three mosquitoes per room. From the 15th week onwards the density in the treated village rose to much the same level as in the control villages, being in the 17th week 6.5 mosquitoes (5.8 vectors) per room, as compared with 5.4 mosquitoes (4.5 vectors) per room in the control villages.

(x) *Batabana* (Fig. 2)

Date of spraying	...	...	...	...	27.9.46
Material used	...	...	...	...	Water-miscible oil mixture containing 0.35 per cent. 'Gammexane'
Percentage of houses sprayed	...	...	...	...	100
" rooms	"	"	...	...	53
Wall surface	...	...	...	...	Mud (5), thatch (1)
Calculated application-rate of 'Gammexane'	...	...	...	...	5-7 mgm. per sq. ft.

The mosquito density was low when this village was sprayed—3 mosquitoes (3 vectors) per room. During the seven weeks after spraying only one anopheline female mosquito was found. For 13 weeks after spraying the density remained for the most part below one mosquito per room, but in the 14th week there was a marked rise to 5.5 mosquitoes (5.0 vectors) per room, as compared with 2.9 mosquitoes (2.6 vectors) in the control villages, and this higher density was maintained over the three following weeks.

### General Conclusions

From the results obtained in the Marampa district it appears that the optimum concentration of 'Gammexane' for use as a residual spray against adult mosquitoes is in the region of 10 mgm. per sq. ft., i.e., a 0.5 per cent. solution of 'Gammexane' applied at the rate of 4 pints per 1,000 sq. ft. Thus an apparent persistence of the insecticide of 18 weeks was produced in Katik and in the labour-lines, and of 27 weeks in Magbenkiti, when 0.5 per cent. 'Gammexane' in kerosene was used at approximately 10 mgm. per sq. ft. The treatment of Batabana with water-miscible oil at 5-7 mgm. per sq. ft. of 'Gammexane' produced an apparent persistent effect of only 13 weeks. The treatment



of areas at a higher application-rate of 'Gammexane,' viz., 11-21 mgm. per sq. ft., produced a persistent effect little different from treatments at 10 mgm. per sq. ft., e.g., 18 weeks in the case of the clerks' quarters and type 'C' quarters and 22 weeks in the case of Mafawki.

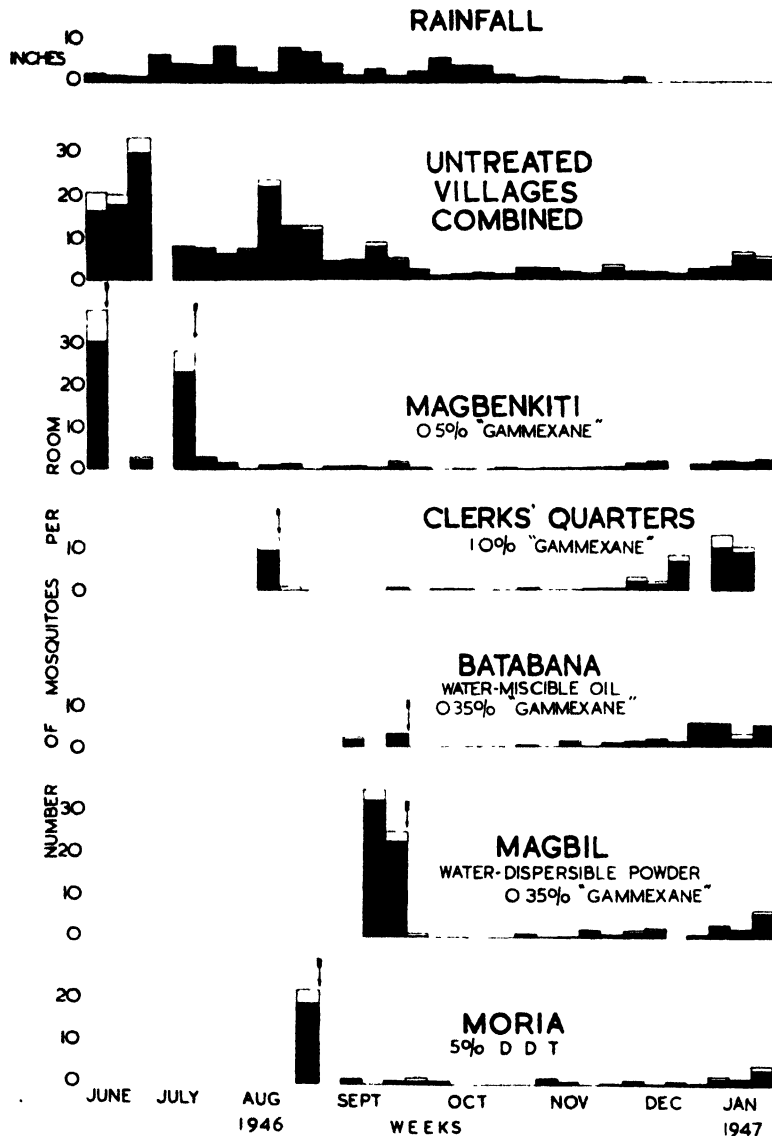


FIG. 2. Rainfall and weekly mosquito room-indices in some of the villages of the Marampa district of Sierra Leone, June, 1946-January, 1947.

There is no apparent difference in effect between the water-miscible oil mixture and the solution of 'Gammexane' in kerosene, but the water-dispersible powder mixture

produces a shorter persistence than the other two. Thus the treatment of Magbil with a water-dispersible powder mixture at 8-17 mgm. of 'Gammexane' per sq. ft. only produced an apparent persistence of 14 weeks. It is suggested that the large amount of goulac in this mixture may have the effect of covering some of the insecticide and so preventing it from being effective, or, alternatively, that the absorption of this mixture is less and the evaporation of the insecticide likely to be greater.

The large variations in the apparent persistences produced after treatments of villages with 0.5 per cent. solutions of 'Gammexane' at the same application-rates of approximately 11 mgm. per sq. ft. can be attributed to several factors. In the first place, the persistence appears to depend on the percentage of rooms in the village treated with the insecticide. A persistent effect of only 3-4 weeks was produced after the treatment of Mafawki (27 per cent. of the rooms treated) and Magbenkiti (40 per cent. of the rooms treated), while in Katik (60 per cent. of the rooms treated) the insecticide appeared to persist for 18 weeks. The spraying of Rochendata (40 per cent. of the rooms) produced an intermediate persistence of approximately 11 weeks, although this is considered an absolute maximum and may be an overestimate. The short persistent effects produced after the initial treatments of Mafawki and Magbenkiti and after the treatment of Rochendata can also be explained by the fact that these were the first villages to be sprayed and that, at the time when they were sprayed, there were 10 other villages or groups of labourers' quarters untreated in the area (the 'boys' quarters were being treated with smoke-generators). Later, when more and more of the whole area was treated, longer apparent persistences were produced. It is therefore suggested that these longer persistences do not necessarily mean that the insecticide persists in its lethal effect for so long, but that the treatment of most of the area has reduced the mosquito-population in that area to such an extent that some time elapses, after the effect of the insecticide has ceased, before breeding recommences at a sufficient rate to produce the pre-treatment level of the mosquito density. This aspect is discussed in more detail in the general conclusion at the end of this report (see below).

The apparent persistent effect of 27 weeks produced after the second spraying of Magbenkiti (54 per cent. of the rooms) with 0.5 per cent. 'Gammexane' at 11 mgm. per sq. ft. might also indicate that some of the insecticide applied at the first spraying was still present and effective after the second spraying, i.e., that the amount of 'Gammexane' per sq. ft. after the second spraying was more than 11 mgm. per sq. ft. Other workers have found that glass surfaces treated with DDT which have lost their insecticidal activity can be reactivated by spraying with a solution of ground-nut oil, and that such reactivated surfaces retain their insecticidal properties for longer periods than residues not containing an oil film (Cawnpore Ordnance Laboratories, 1945b).

#### *Treatment of a Small Village in the Marampa Area with DDT (Fig. 2)*

Moria, a small village of six houses in the Marampa area, was sprayed on September 2nd, 1946, with a 5 per cent. solution of DDT in kerosene. The calculated application-rate was 80 mgm. of DDT per sq. ft. (assuming 70 per cent. purity of the compound). All the houses were sprayed, the percentage of rooms sprayed being 61.

The room density immediately before spraying was 22 mosquitoes (19 vectors) per room, and for 20 weeks afterwards it remained for the most part less than one mosquito

per room. In the 21st week the density rose to 4·8 mosquitoes (3·8 vectors) per room, when the density in the control villages was 5·4 mosquitoes (4·5 vectors) per room.

Thus DDT, at a much higher concentration, produced, under similar conditions, a very similar persistent effect to 'Gammexane' at a much lower concentration.

### *Wellington*

The comparatively large village of Wellington, comprising some 200 houses, was divided into seven areas for the purpose of residual spraying with different types and concentrations of 'Gammexane.' Six of these areas in the main part of the village were parallel series of from 15 to 18 houses, extending from the Freetown-Hastings road down to the coast (map 1). The seventh area of 11 houses was in the part designated Wellington-East, the eastern part of the village on the inland side of the road. Untreated houses in and between these areas served as controls.

The different treatments given were: 0·33 per cent. and 0·5 per cent. 'Gammexane' in kerosene, a water-dispersible powder mixture containing 0·5 per cent. 'Gammexane,' and solutions of benzene hexachloride in kerosene containing 0·12 per cent., 0·22 per cent., 0·35 per cent. and 0·37 per cent. 'Gammexane.' Application-rates varied from 1·2 mgm. to 10·21 mgm. of 'Gammexane' per sq. ft.

Analysis of the results of treatment of all the areas revealed that the mosquito densities were lowered, in most cases considerably, over the period of observation (8-9 weeks), but no conclusion could be reached as to the relative persistence of the various types and concentrations of 'Gammexane.' Treatment of about half the houses in the village also had the effect of lowering the mosquito density in the untreated control houses. In the week before any spraying was done the mosquito density in the village was 38·3 mosquitoes (32·9 vectors) per room. Two weeks after six of the areas had been sprayed (and after Wellington-East had been 'smoked'), the mosquito density in the control houses was only 4·3 mosquitoes (3·4 vectors) per room. It is almost certain, therefore, that the treatment of one area with one type and concentration of 'Gammexane' would have an effect on the mosquito density in the adjacent areas treated with other types and concentrations of 'Gammexane,' and vice versa.

### THE EFFECT OF TREATMENT ON THE SPOROZOITE-RATE

#### *Villages South of Freetown*

Of 60 *A. gambiae* and three *A. melas* from Sussex and No. 2 River, dissected 2-8 weeks after treatment, none were found with infected salivary glands.

Eleven *A. gambiae* from the control villages of Ogo Farm and Baw-Baw proved negative for sporozoites on dissection.

#### *Marampa and District*

One hundred and forty-nine *A. gambiae* were dissected from the 'boys' quarters, Rochendata, Mafawki, the labour-lines and type 'C' quarters, 2-10 weeks after treatment, and four were found with infected salivary glands. Two of these, however, were taken from Rochendata 10 weeks after treatment, when it was considered that the effect of the 'Gammexane' was disappearing. One hundred and twenty-eight *A. gambiae* and

one *A. funestus* were dissected from Rokontah, Rogbanch, Batabana, Moria, Lunsar and the clerks' quarters—all control villages or villages before treatment—and seven *A. gambiae* were found with infected salivary glands.

### Wellington

Sixteen *A. gambiae* and three *A. funestus* were dissected from Wellington before treatment, and none were found with infected glands.

Twenty-seven *A. gambiae*, one *A. melas* and seven *A. funestus* were dissected from Wellington and Wellington-East two weeks after treatment, and one *A. gambiae* was found with infected salivary glands.

Thus the sporozoite-rate obtained from dissections of 247 mosquitoes from treated villages was 2 per cent. (if the two infected mosquitoes from Rochendata are excluded), and that from the dissection of 159 mosquitoes from untreated areas was 4.4 per cent.

The numbers of mosquitoes dissected are too small to give reliable sporozoite-rates, but indications are that the effect of treatment was to lower the sporozoite-rate, though infected mosquitoes were not entirely eliminated from treated areas.

### CONCLUSION

It is concluded from the trials carried out in Sierra Leone that 'Gammexane' in the form of a residual spray is an efficient means of reducing the population of mosquitoes in houses, and indications are that a 0.5 per cent. solution of the insecticide, applied at the rate of 10 mgm. per sq. ft. and sprayed on to every available internal wall surface of all the houses in an area, will reduce the mosquito-population in the houses to almost nil for a period of about six months.

Throughout these trials it was repeatedly noticed that treatment of an area with 'Gammexane' lowered the mosquito density not only in that area, but also in adjacent areas. A similar indication has been found in areas treated with DDT. Trapido (1946), working on the control of *A. albimanus* in the Panama region by residually spraying houses with DDT, used as control catching-stations three horse-baited traps, one in the treated village itself and the other two at distances of 300 ft. and 900 ft. respectively from the treated village. He found considerable reductions in the mosquito-populations in the trap in the village and in the one 300 ft. away, after the village had been sprayed, but no obvious reduction in the trap 900 ft. distant. He concluded, therefore, that the range of effectiveness of the insecticide was less than 900 ft. And Gahan and Lindquist (1945), in America, after spraying houses with DDT as a control measure against *A. quadrimaculatus*, found a significant reduction in the breeding of the species in adjacent rice-fields, viz., 57 per cent. and 63 per cent. in lightly and more heavily treated areas respectively.

In Sierra Leone it was not possible, in the short time of the trials, to investigate the effect of treatment on the intensity of breeding, as circumscribed persistent breeding-places with sufficient numbers of larvae to give significant results were very difficult to find. However, analysis of the changes in the mosquito-populations both in treated and in untreated areas provides strong indications that the breeding in and around the treated

areas was considerably reduced. It is proposed to discuss this evidence in detail at this point.

*b The Effect of Treatment on the Mosquito-Population of Untreated Houses Within a Treated Village*

Analysis of all the results in all the areas used for trials in Sierra Leone shows consistently that the mosquito density in the untreated houses in a treated village a few weeks after treatment was much the same as in the treated houses, and remained so. As a rule the density in the untreated houses, for three or four weeks after treatment, remained higher than in those treated, but after that time the density fell to the level in the treated houses and remained at that level. In most of the villages under observation only a few untreated houses were examined, and it is proposed to use results obtained in the labour-lines at Marampa as an example, since larger numbers of untreated houses were examined in this area than elsewhere. Here a total of 47 per cent. of the rooms were sprayed. Table XII shows the room densities in treated and untreated rooms in this area for 21 weeks after treatment. During the week before treatment the mosquito density was 7.5 mosquitoes (7.0 vectors) per room. For 18 weeks after treatment the mosquito density in the treated rooms remained for the most part at less than one mosquito per room, and in the 19th, 20th and 21st weeks increased to 2-3 mosquitoes per room. During the first four weeks after treatment the density in the untreated rooms varied from two to seven mosquitoes per room, but in the fifth week it was down to nil and in the 13 weeks following it seldom rose to more than one mosquito per room. In the 19th, 20th and 21st weeks after treatment the density increased again as in the treated rooms.

Similar reductions in the mosquito densities in untreated houses occurred in Wellington after about half the village had been treated with 'Gammexane.'

*The Effect of Treatment on Adjacent Untreated Villages*

*Villages on the South-West Coast of the Colony of Sierra Leone*

The peak in the mosquito density in the uncontrolled villages on the south-west coast of the Colony of Sierra Leone occurred in the week June 16th-22nd, 1946, and amounted to 71 mosquitoes (67 vectors) per room. Before this only two of the villages under observation had been sprayed (one of them twice). By July 8th, 1946, four of the seven villages had been sprayed with 0.5 per cent. 'Gammexane' in kerosene, and in succeeding weeks the densities in the untreated villages combined were as follows.

Week	No. of mosquitoes per room	
	Total	Vectors
July 7th-13th ...	18.3	15.0
" 14th-20th ...	12.2	8.9
" 21st-27th ...	4.6	4.1
Aug. 28th-Sept. 3rd	2.1	1.6
Sept. 4th-10th ...	0.9	0.5

Thus in five weeks the mosquito-population decreased from 18 per room to less than one per room, and it remained around this low level until observations ceased at the end

of October, 1946. It will be remembered that these seven villages all occur within an area six miles in extent, and that the untreated villages lie within half a mile to one mile of the nearest treated village.

In the village of Wellington, about eight miles north-east of these villages, the mosquito density was comparatively high when observations were started at the end of August, and continued to be high in the untreated houses for some four weeks after treatment of about half the village with 'Gammexane.'

Week	No. of mosquitoes per room	
	Total	Vectors
Aug. 25th-31st ...	38.3	32.9
Sept. 1st-7th ...	23.0	18.9
" 8th-14th ...	35.5	20.7
" 15th-21st ..	4.3	3.4
" 22nd-28th ...	11.1	8.4
Oct. 29th-Nov. 5th	4.8	3.4
Nov. 6th-12th ...	2.1	1.1

In view of the fact that the mosquito density in another part of the Colony was still high in September, it was decided to examine previous years' records for two villages in the Colony, Aberdeen and Wellington (map 1), to find out the natural variation in the mosquito densities over a whole year.\* Table X shows the monthly room densities in Aberdeen in 1941 and 1942, and in Wellington in 1944 and 1945. Some control measures were being effected in these villages in those years, but they are considered not to have affected the general monthly distribution of the mosquito densities. It can be seen that the densities in every case rise steeply in June, but remain high until at least September and often until October. It is therefore concluded that the treatment of four of the seven villages on the south-west coast of the Colony produced a marked drop in the mosquito density over the whole area, including the untreated villages, from August onwards.

#### *Marampa and District*

From mid-June to the end of September, 1946, the monthly mosquito densities in untreated villages combined varied from four to 33 mosquitoes per room. By the end of September 11 of the 14 villages in this area had been treated with 'Gammexane,' and from then until the end of December the density never rose above 3.5 mosquitoes per room and for the most part remained between one and two mosquitoes per room. In the same period in 1945, i.e., October to December, the room densities (total mosquitoes) in several of the villages were:

Mafawki ...	...	7-8
Magbenkiti ...	...	6-7
Rochendata ...	...	5-7
Labour-lines ...	...	5-7
'Boys' quarters ...	...	3-5

Moreover, mosquito-catches carried out in September, 1946, in 29 of the kitchen store-rooms of the European bungalows (untreated) yielded a room-index of 0.5 total

Information was kindly provided by Dr. G. A. Walton, Government Entomologist, Sierra Leone.

mosquitoes (0·4 vectors), while in September, 1945, the corresponding figures were 7 (6).

Twelve of the Marampa villages are all situated within an area of four square miles, including two of the untreated villages, and it is again concluded that treatment has lowered the mosquito density over the whole area.

All this evidence points to the fact that the residual spraying of houses with 'Gammexane' in an area where the malaria-carrying mosquitoes are predominantly house-haunting species lowers the mosquito density over the whole area, presumably by reducing the intensity of breeding, and it seems conceivable that if this method were to be used thoroughly in an isolated area, not subject to invasion by mosquitoes from other areas, complete eradication of these mosquitoes would be effected without any resort to larval-control measures. This would be of particular importance in the case of areas infested with *A. gambiae*, a species which breeds in all kinds and sizes of collections of water and which is difficult to eradicate by larval-control measures alone.

### SUMMARY

1. Three areas in Sierra Leone were chosen for trials with 'Gammexane' as a control measure against adult *A. gambiae*, *A. melas* and *A. funestus* in houses.

2. Portions of these areas were left untreated as controls, and mosquito-catches made in treated and untreated portions were compared.

3. Trials with smoke-generators containing 'Gammexane' did not prove very successful, owing to the inadequate retention of the smoke by the houses. Some control was effected for from one to three weeks after 'smoking.'

4. Trials with residual sprays containing 'Gammexane' proved much more successful, and very significant reductions in mosquito densities in treated houses were maintained over periods varying from one to six months, the length of the periods depending on the rate of application of the insecticide, the thoroughness of treatment of the houses, and the proportion of the area treated.

5. The insecticide was used in various forms, viz., solutions of 'Gammexane' in kerosene, water-dispersible powder mixtures, water-miscible oil mixtures and solutions of crude benzene hexachloride in kerosene. These were in concentrations varying from 0·12 per cent. to 1 per cent. 'Gammexane,' and application-rates varied from 1 to 20 mgm. of 'Gammexane' per sq. ft. of wall surface.

6. It is concluded that a solution or mixture containing 0·5 per cent. 'Gammexane' applied at the rate of 10 mgm. 'Gammexane' per sq. ft. (i.e., 4 pints of the solution or mixture to 1,000 sq. ft.), and sprayed on to all the internal wall surfaces of all the houses in an area, will reduce the mosquito density in these houses to almost nil over a period of about six months.

7. Small numbers of dissections of mosquitoes from treated and untreated areas showed a lower sporozoite-rate in the treated areas.

8. Evidence is produced to show that the treatment of houses in part of an area with 'Gammexane' reduces the mosquito-population not only in that part, but also in adjacent parts 1-2 miles distant.

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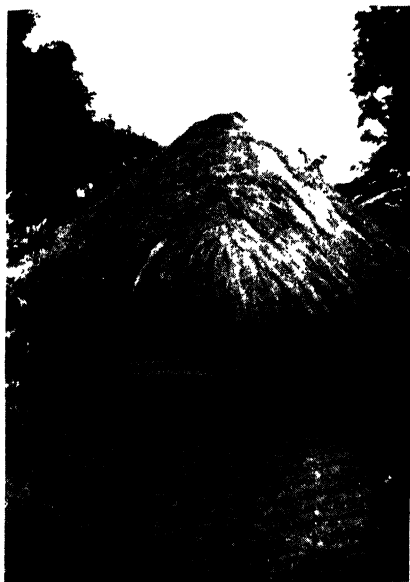


FIG. 1. Sussex: single-storied house with wooden walls and a thatch roof



FIG. 2. Sussex: two-storied house with wooden walls and a corrugated-iron roof



FIG. 3. Sussex: two-storied house made completely of corrugated iron.



FIG. 4. Sussex: single-storied house with wooden walls and a corrugated-iron roof.





FIG 5 Wellington : house with mud walls and a corrugated-iron roof.

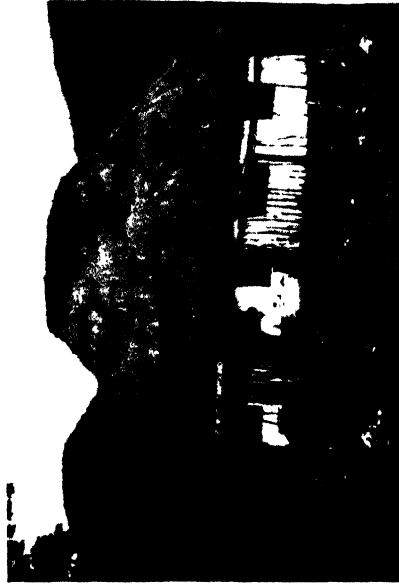


FIG. 6. Public Works Department compound : house with mud walls and a thatch roof.



FIG. 7. Katik : typical houses of a native village, with round mud walls and a conical thatch roof.



FIG 8 Rochendata : rectangular type of house, with mud walls and a corrugated-iron roof.

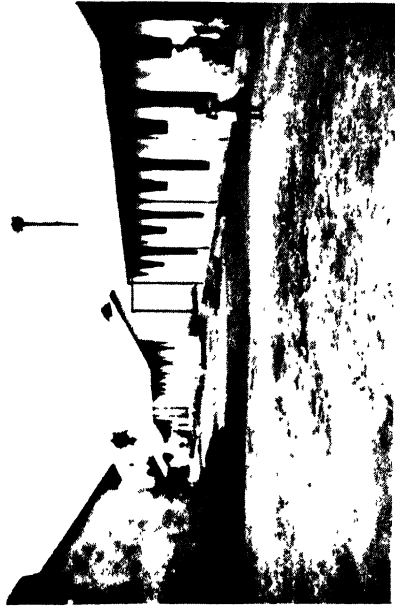


FIG. 9 'Boys' quarters: some of the blocks, each containing five rooms



FIG. 10 'Boys' quarters: a single block of rooms, showing the whitewashed concrete walls and corrugated-iron roof

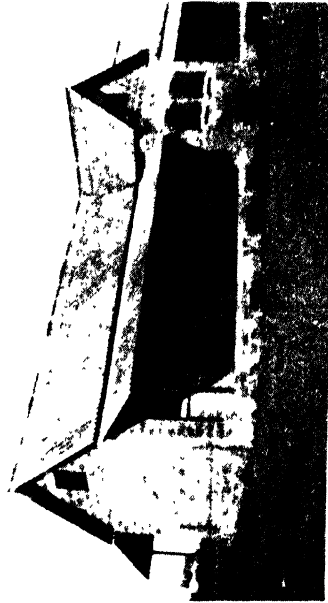


FIG. 11. Type 'C' quarters: one of the six blocks of rooms, each containing six bedrooms and a central 'parlour'



FIG. 12 Labour-lines: one of the four-bedroomed mud houses, with mud walls and a thatch roof. The concrete blocks of rooms in this area can be seen behind this house



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Similar data for the Marampa area were kindly provided by Dr. Smeaton, of the Sierra Leone Development Company. Figures on the mosquito densities in the Marampa area for the year 1945 are published by permission of that Company.

This work was carried out under the supervision of Professor G. Macdonald, Director of the Ross Institute, to whom I am indebted for advice and criticism.

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# FIELD TRIALS WITH 'GAMMEXANE' AS A MEANS OF MALARIA CONTROL BY ADULT MOSQUITO DESTRUCTION IN SIERRA LEONE\*

## II.—THE EFFECT OF TREATMENTS OF HOUSES WITH 'GAMMEXANE' ON THE MALARIA-RATE IN THE INHABITANTS

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### GENERAL METHOD

To determine whether treatment with 'Gammexane' of villages on the south-west coast of the Colony of Sierra Leone and in the Marampa district of the Protectorate of Sierra Leone would affect the malaria incidence in the inhabitants, blood films were taken—for the most part from children between the ages of two and 10 years—at the commencement of the trials and again some five or six months later, both from the villages which were treated with 'Gammexane' and from those which were left untreated. As no marked decrease in the parasite-rate was expected in so short a time, parasite counts (see below) were done on all these films, in an attempt to determine whether transmission had ceased or had been reduced in the treated villages. Blood films were also taken from babies born after the commencement of the treatments, from both treated and untreated villages.

Children were examined from all the villages under observation on the south-west coast of the Colony of Sierra Leone, but from only five treated villages and one untreated village in the Marampa district.

Thick blood films were made by the fowl-cell technique of Christophers, Sinton and Covell (1931), mixing equal volumes of blood and of a fowl-cell suspension containing 20,000 fowl cells per c.mm. In the case of the babies ordinary thick films were taken.

In counting the parasites a restricted field was used, and the average number of fowl cells per field was found to be 10. The number of parasites counted in 50 fields (i.e., to 500 fowl cells) multiplied by 40 was taken as the number of parasites per c.mm. of blood. One hundred fields were examined before a film was recorded as negative. Where very large numbers of parasites were present, the number in only 10 or 20 fields was counted and the calculation was modified accordingly.

In the case of the ordinary thick blood films taken from babies, the films were examined for the presence or absence of parasites only.

Most of the parasites found were *Plasmodium falciparum*, but several mixed infections of *P. falciparum* and *P. malariae* were recorded and a few infections of *P. malariae* alone. Gametocytes were rarely found and are not separated in the rendering of the results.

\* This work was made possible by a grant from Imperial Chemical Industries Limited.

In Tables I and II the children have been divided into two age-groups—five years and under, and over five years. The numbers of parasites per c.mm. have also been grouped, arbitrarily, as follows :

0- 500 per c.mm	
500-1,000	"
1,000-3,000	"
Over 3,000	"

## RESULTS OF EXAMINATIONS OF BLOOD FILMS FROM CHILDREN BETWEEN THE AGES OF TWO AND TEN YEARS

VILLAGES ON THE SOUTH-WEST COAST OF THE COLONY OF SIERRA LEONE (Table I)  
*Treated Villages*

1. *Sussex and Sherbro Town.* Before the treatment of these villages the parasite-rate was 76.8 per cent. Eighteen weeks after spraying the houses with 0.5 per cent. 'Gammexane' in kerosene (23 weeks after 'smoking,' which was considered more or less ineffective in reducing the mosquito-population), the parasite-rate was still 73.9 per cent. and the number of higher infections had increased, even though, in this second examination, the proportion of children over five years of age was greater.

TABLE I

Parasite-rates and parasite counts in the villages on the south-west coast of the Colony of Sierra Leone before and after treatments with 'Gammexane'

Locality	No. examined	Age- distribution		No. positive	Parasite- rate %	Parasite-count distribution (no. per c.mm.)			
		5 years and under %	Over 5 years %			0-500 %	500- 1,000 %	1,000- 3,000 %	Over 3,000 %
VILLAGES BEFORE TREATMENT									
Sussex and Sherbro Town	82	48	52	63	76.8	70	10	10	10
Lakka ... ..	17	59	41	13	76.5	77	0	0	23
No. 2 River ... ..	22	59	41	20	90.9	50	10	25	15
Hamilton ... ..	21	0	100	19	90.5	95	0	5	0
Ogoo Farm ... ..	17	18	82	15	88.2	53	20	20	7
Baw-Baw ... ..	30	60	40	24	80.0	42	8	8	42
Total . . . . .	189	44	56	154	81.5	65	9	11	15
VILLAGES AFTER TREATMENT									
<i>Treated Villages</i>									
Sussex and Sherbro Town	69	25	75	51	73.9	66	4	18	12
Lakka ... ..	23	74	26	21	91.3	24	19	19	38
No. 2 River ... ..	19	68	32	17	89.5	35	12	24	29
Total ... ..	111	42	58	89	80.2	50	9	20	21
<i>Untreated Villages</i>									
Hamilton ... ..	36	14	86	31	86.1	45	36	6	13
Ogoo Farm ... ..	9	78	22	8	88.9	25	12.5	12.5	50
Baw-Baw ... ..	23	48	52	21	91.3	38	38	24	0
Total ... ..	68	34	66	60	88.2	40	34	13	13

2. *Lakka*. The parasite-rates before and after treatment were 76·5 per cent. and 91·3 per cent. respectively, the latter being 22 weeks after the first spraying of the houses with 'Gammexane.' The proportion of higher intensities of infections had also increased considerably after treatment. A slightly higher proportion of children under five years of age was examined on the second occasion.

3. *No. 2 River*. Almost the same parasite-rate was obtained before treatment (90·9 per cent.) as 16 weeks after treatment (89·5 per cent.), but the parasite intensity had increased significantly. A slightly higher proportion of children under five years of age was examined on the second occasion.

### *Untreated Villages*

4. *Hamilton*. Parasite-rates were little different in May (90·5 per cent.) and October (86·1 per cent.), but in October the intensity of the infections was considerably higher. This would be partly accounted for by the greater proportion of children of five years of age and under examined on the second occasion.

5. *Ogoo Farm*. Almost the same parasite-rates were obtained in May and October—88·2 per cent. and 88·9 per cent. respectively—but there was a marked increase in the higher infections. This would again be partly accounted for by the much higher proportion of children in the lower age-group examined on the second occasion.

6. *Baw-Baw*. A slightly higher parasite-rate (91·3 per cent.) was obtained in November than in May (80·0 per cent.). Parasite counts in the groups 500–1,000 and 1,000–3,000 per c.mm. had increased on the second examination, but in the highest group—over 3,000 per c.mm.—had decreased to nil.

Summarizing the results for the coastal area of Sierra Leone, it was found that the four villages which were treated with 'Gammexane' had a combined parasite-rate of 79·3 per cent. before treatment and of 80·2 per cent. 16–22 weeks after treatment, with a greater proportion of infections with high parasite counts (1,000–3,000 and over 3,000 parasites per c.mm. of blood). In the three untreated villages the parasite-rates at the same times were 85·3 per cent. and 88·2 per cent. respectively, with a slight decrease in infections of over 3,000 parasites per c.mm. but with a marked increase in infections of 500–1,000 parasites per c.mm. on the second examination.

It is concluded that both the parasite-rate and the parasite intensity increase naturally, to a small extent, after the rainy season (June to September), and that treatment has had little or no effect on the parasite-rate or intensity in the short time over which observations were made.

## VILLAGES IN THE MARAMPA DISTRICT (Table II)

### *Treated Villages*

1. *Magbenkiti*. The parasite-rate before treatment was 84·4 per cent., and 18 weeks after treatment 76·5 per cent. The parasite intensity also decreased slightly after treatment, chiefly in the group 500–1,000 parasites per c.mm. and to a less extent in the over 3,000 per c.mm. group.

2. *Mafawki*. Sixteen weeks after treatment the parasite-rate had decreased from 92·6 per cent. to 79·3 per cent., accompanied by a significant drop in the number of

infections of over 1,000 parasites per c.mm., in spite of the higher proportion of children in the lower age-group examined on the second occasion.

3. *Rochendata*. The most marked drop of all the villages examined was produced in Rochendata, where the parasite-rate decreased, 16 weeks after treatment, from 96.6 per cent. to 62.1 per cent., accompanied by a marked decrease in the parasite intensity.

4. *'Boys' Quarters*. The parasite-rates before treatment and 14 weeks after treatment with 0.5 per cent. 'Gammexane' in kerosene (18 weeks after 'smoking,' which produced little effect on the mosquito density) were 64.3 per cent. and 60.9 per cent. respectively. On the second examination parasite counts of over 1,000 per c.mm. had increased slightly.

TABLE II

Parasite-rates and parasite counts in the villages in the Marampa district of Sierra Leone before and after treatments with 'Gammexane'

Locality	No. examined	Age- distribution		No. positive	Parasite- rate %	Parasite-count distribution (no. per c.mm.)			
		5 years and under %	Over 5 years %			0-500 %	500- 1,000 %	1,000- 3,000 %	Over 3,000 %
VILLAGES BEFORE TREATMENT									
Magbenkiti . . . . .	32	50	50	27	84.4	41	26	15	18
Mafawki . . . . .	27	19	81	25	92.6	44	8	40	8
Rochendata . . . . .	29	76	24	28	96.6	39	11	39	11
'Boys' quarters . . . . .	14	43	57	9	64.3	56	33	0	11
Labour-lines . . . . .	53	45	55	43	81.1	51	23	12	14
Lunsar . . . . .	67	9	91	53	79.1	74	13	11	2
Total . . . . .	222	36	64	185	83.3	54	17	19	10
VILLAGES AFTER TREATMENT									
<i>Treated Villages</i>									
Magbenkiti . . . . .	34	56	44	26	76.5	61	12	15	12
Mafawki . . . . .	29	62	38	23	79.3	44	35	17	4
Rochendata . . . . .	29	76	24	18	62.1	78	11	11	0
'Boys' quarters . . . . .	23	43	57	14	60.9	65	14	7	14
Labour-lines . . . . .	53	72	28	41	77.4	46	14	20	20
Total . . . . .	168	64	36	122	72.6	56	17	16	11
<i>Untreated Village</i>									
Lunsar . . . . .	51	10	90	36	70.6	91	6	3	0

5. *Labour-Lines*. There was very little difference between the parasite-rates before treatment (81.1 per cent.) and 12 weeks after treatment (77.4 per cent.). The slight increase in the parasite intensity after treatment would be partly accounted for by the greater proportion of children in the lower age-group examined on this occasion.

#### *Untreated Village*

6. *Lunsar*. Parasite-rates of 79.1 per cent. and 70.6 per cent. respectively were obtained in July and October, with fewer infections of over 500 parasites per c.mm. in the latter month.



It would thus appear that the malaria incidence in the Marampa district is higher at the beginning of the rainy season than at the end, that the decreases in the parasite-rates produced in the treated villages would be to some extent natural, and that treatment with 'Gammexane' has produced little or no effect in the short time over which the observations were made. The more marked decreases obtained in the villages of Mafawki and Rochendata might to some extent be due to a reduction in malaria transmission following treatments of the houses with 'Gammexane.'

#### RESULTS OF EXAMINATION OF BLOOD FILMS FROM BABIES BORN AFTER TREATMENTS HAD COMMENCED

Only small numbers of babies born during the trials were available for examination, especially in the coastal villages. Great difficulty was experienced in assessing the correct ages of the infants, and those supposedly up to six months old were examined.

Of seven babies examined from the treated villages of the coastal area, three were found with malaria parasites. Of five examined from the untreated villages in this area, two were found with malaria parasites. In the Marampa area 17 of the 28 babies examined from treated villages had malaria parasites in their blood, and seven of the 13 babies from the untreated village of Lunsar.

These figures indicate that transmission was occurring to much the same degree in the treated villages as in the untreated ones.

#### CONCLUSIONS

In the short time over which observations were made, which varied from 12 to 22 weeks after treatment with residual sprays containing 'Gammexane,' little or no difference in the parasite-rates and intensities was produced in two areas in Sierra Leone. However, it is highly probable that infections obtained at, or just before, the commencement of the trials (and spraying was carried out in most of the villages in these areas when the mosquito density was at its height) would not have had time to die out in such a short period, and it is considered that further examinations over a longer period of time (at least one year) would be necessary to determine whether the treatment of houses with 'Gammexane' would lower the malaria incidence in the local population. Moreover, an important factor, which would significantly alter the results, has not been taken into account, namely, the movement of the populations between treated and untreated villages, which in both areas are very close to one another.

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# THE CONSUMPTION OF HAEMOGLOBIN BY MALARIA PARASITES

BY

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Evidence of the consumption of haemoglobin by malaria parasites during their growth in red cells has previously been derived from three sources. The pallor of infected red cells, containing, for example, the larger forms of *Plasmodium vivax*, has been supposed to be due to loss of haemoglobin from the red cell. The formation of malarial pigment and its identification with haematin indicate that the parasites utilize haemoglobin. Observations on the respiration of *P. knowlesi* (Christophers and Fulton, 1938) have shown that haemoglobin is altered by that parasite, if it is not actually split up.

The present investigation of the subject is based on the staining reaction of cyanol with haemoglobin, applied to thin blood films made from the malarial blood of human patients, monkeys and fowls. The changes in the haemoglobin content and its distribution in infected cells were observed at various stages of growth of the parasites. The parasites studied were *P. vivax*, *P. falciparum*, *P. knowlesi* and *P. cathemerium*.

## METHOD

Cyanol (colour-index 715), in the reduced and colourless state, is oxidized in the presence of haemoglobin, which it stains blue. Dunn (1946) described a method of staining haemoglobin in sections. This technique, applied to blood films fixed with methyl alcohol, causes a rapid colour-change, but the blue-stained haemoglobin is washed out of the red cells. Investigation has shown that this is due to Dunn's working reagent being too acid for these films, and the following procedure for thin blood films has been evolved from his method of staining sections.

The stock solution is prepared as described by Dunn: 1 gm. of cyanol is dissolved in 100 ml. of distilled water; to this is added 10 gm. of zinc powder (C.P.) and 2 ml. of glacial acetic acid; the mixture is then boiled and quickly loses its blue colour.

The working stain is made up *immediately* before use, and consists of 5 ml. of filtered stock solution (which has been kept over the zinc) plus 0.5 ml. of 3 per cent. hydrogen peroxide.

Thin blood films are fixed with methyl alcohol and allowed to dry. The stain is then run on to the film and a blue-green colour is seen gradually to develop within a minute or two. When this colour-change has developed the films are gently washed with tap-water and counterstained. If a stock solution has been allowed to stand for a couple of weeks it may require acidification before use: one drop of glacial acetic acid added to 5 ml. of filtered stock solution is usually sufficient for this purpose.

The counterstain used in these experiments was either 1 per cent. aqueous safranin or Leishman's stain diluted as for thin blood films. The various species of plasmodia differ in their staining reactions with safranin—*P. gallinaceum*, for instance, stains pink,

\* Working under a grant from the Medical Research Council.

whereas *P. vivax* is coloured much more lightly. When Leishman's stain is used as the counterstain there is no eosinophilic staining of the red cell, as it retains its cobalt green colour. The parasite does not stain so well after this preliminary staining as with the ordinary Leishman stain.

The picture obtained with this stain is as follows: normal red cells stained cobalt green, and parasites stained variously pink (with safranin in *P. gallinaceum* infections), a faint brownish-yellow (with safranin in *P. vivax* infections), or blue-red (with Leishman's stain in *P. vivax* and *P. falciparum* infections). The nuclei of fowl red cells stain a warm yellow-brown with safranin. The malarial pigment is prominent but does not appear to react definitely with cyanol under the conditions here described.

## RESULTS

Plate X shows the appearance of red cells invaded by various malaria parasites and the changes seen with different stages of development of these parasites.

Young forms, such as the small rings and appliqué forms of *P. falciparum*, are not associated with any apparent loss of cyanol-staining material from the containing red cell. Large rings, on the other hand, show loss of green coloration from their immediate neighbourhood, so that there is no green colour to be seen through the vacuole.

If amoeboid activity develops, as is seen notably with the older forms of *P. vivax*, the parasite lies in a red cell which shows only a small amount of green coloration. The remaining green colour is irregularly distributed throughout the enlarged red cell, and is seen in those areas not immediately in contact with the parasite. In infections with *P. gallinaceum*, a plasmodium which exhibits little amoeboid activity, the green stain is absent only from that part of the red cell occupied by the parasite.

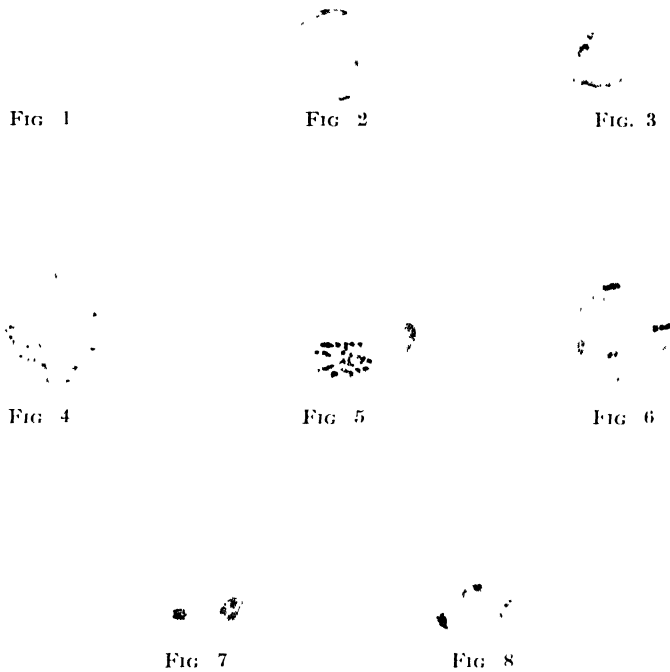
Red cells containing the fully grown forms of parasites, such as the gametocytes of *P. vivax* or *P. falciparum* and the mature schizonts of *P. knowlesi*, show little, if any, staining with cyanol. The stroma of the rest of the red cell around the parasite is stained with the counterstain.

## DISCUSSION

Cyanol is a diaminotriphenylmethane which loses its blue colour with hydrogenation. In the presence of haemoglobin and readily available oxygen in an acid medium the leuco-compound regains its colour.

In the normal red cell the haemoglobin appears to be distributed evenly throughout the red cell, judging from the results obtained with this stain. Text-book descriptions of red cells containing, for example, the amoeboid forms of *P. vivax* give a picture of a red cell like a bladder, containing haemoglobin in a simple solution, and indicate that the pallor seen in these cells is due to a general rather than to a local reduction in the amount of haemoglobin in the cell. There is no evidence that the Romanowsky stains specifically give a red colour with the haemoglobin in the red cell. The stromata of red cells in thin films, in which the red cells have been laked with water, give an eosinophilic reaction with Leishman's stain. Similar films treated with this cyanol stain do not give a blue colour.

Warasi (1927) pointed out that the red cells harbouring some species of the parasite of human malaria were pale, whereas those containing parasites of other species in the same stage of development were not. The films were stained by one of the Romanowsky stains. Sinton and Ghosh (1934) suggested that the Schüffner's dots in the infected red



*Robert Black*

- FIG. 1. Normal human red cell stained with cyanol and Leishman.  
FIG. 2. Large ring and appliqué forms of *P. fulciparum* (cyanol and Leishman).  
FIG. 3. Amoeboid form of *P. irax* (cyanol and Leishman).  
FIGS. 4 and 5. Gametocytes of *P. irax* and *P. fulciparum* (cyanol and Leishman).  
FIG. 6. Schizont of *P. knowlesi* (cyanol and Leishman).  
FIGS. 7 and 8. Forms of *P. gallinaceum* (cyanol and safranin).

*Note.*—Red cells are not accurately drawn to scale.



cells of vivax malaria might be local aggregations of haemoglobin derivatives, and that this might contribute to the pallor of the red cells containing plasmodia. Lawson (1913) described reduction in the red coloration of erythrocytes in the area immediately adjacent to malaria parasites. The results of the experiments described here show that the actively amoeboid and more slowly growing parasites use the haemoglobin from most of the red cells, whereas the relatively inactive species merely use that in their immediate neighbourhood.

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# THE INFECTION OF RATS BY TRYPANOSOMES (*T. RHODESIENSE*) TAKEN FROM MAN EARLY IN THE DISEASE

BY

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In 1944 and 1945, while testing the infectivity to man of the Tinde strain of *Trypanosoma rhodesiense*, it was found that rats inoculated with human blood containing trypanosomes did not necessarily become infected (Fairbairn and Burt, 1946). The investigation was continued during 1946, and the results for the three years are summarized in Table I.

TABLE I

The results of the inoculation into rats of human blood containing *T. rhodesiense*

Volunteers infected from	Rats inoculated with blood from			
	Arm-reaction		Blood	
	No. positive	No. negative	No. positive	No. negative
Thomson's gazelle line ... ..	18	2	40	6
Monkey line ... ..	20	1	45	3
Sheep " ... ..	10	36	41	57
Reedbuck ex sheep ... ..	0	2	8	4
Volunteer 280 (himself infected from T.G. line) ...	9	13	15	0

The history of these lines is briefly as follows.

**Sheep Line.** The original strain of *T. rhodesiense* was isolated from man in October, 1934 (Corson, 1936), and has since been passaged through sheep only. This strain is now showing *gambiense* characteristics (Fairbairn and Burt, 1946).

**Thomson's Gazelle Line.** In November, 1936, a line was taken off the sheep line and passaged through a variety of antelope. Since March, 1940, it has been passaged through Thomson's gazelle exclusively.

**Monkey Line.** In March, 1940, a line was taken off the sheep line and has been passaged since then through *Cercopithecus* monkeys.

All passages have been by cyclically infected *Glossina morsitans*. For full details of these lines the reader should consult Fairbairn and Burt (1946).

The following technique in the inoculation of rats was used.

**Arm-Reaction.** The arm-reaction, produced by the bite of an infected *G. morsitans*, was punctured daily and a stained thick film was examined. Either on the same or on the following day that trypanosomes were first seen, the arm-reaction was repunctured, a thick and a thin film were made, and drops of blood were drawn up into a 1.0 c.cm. syringe filled with citrated saline until the blood appeared in the barrel of the syringe. The blood and citrated saline were well mixed, and 0.5 c.cm. was inoculated subcutaneously

into each rat. (As the same syringe and needle were used on each occasion approximately the same amount of blood was withdrawn from each patient.) Examination of the thick film confirmed the presence of trypanosomes, and the morphology of the trypanosomes was studied in the thin films.

**Blood.** On the day that trypanosomes were first found in a stained thick film of the patient's blood, or on the following day, a thin film was made, and blood was then withdrawn from the antecubital vein into a dry sterile syringe, and 1.0 c.cm. of the undiluted blood was immediately inoculated subcutaneously into each of several rats.

In the case of volunteers 300 and 334 (Table II), who had infections of a relapsing character, rats were again inoculated from the arm-reaction, and with blood, whenever trypanosomes reappeared. The disease in volunteer 334 was almost avirulent, although he had been infected from volunteer 280 (himself infected from the Thomson's gazelle line) who had an acute attack (Fairbairn and Burt, 1946).

TABLE II  
The infections in rats, and the men from whom they were inoculated

	Rats inoculated from	Volunteer infected from	Result of rat inoculations	Incubation-period in infected rats
Group I	Volunteer 280	Thomson's gazelle line	All 19 rats infected	4-8 days
	" 286	" "		
	" 314	Monkey line		
	" 323	" "		
	" 327	Volunteer 280		
" 333	" 280			
Group II	Volunteer 302	Sheep line	All 14 rats infected	9-18 days
	" 326	Volunteer 280		
	" 334	" 280		
	(on three different days)			
Group III	Volunteer 300 (twice)	Sheep line	9 rats infected	8, 10, 14, 16, 21, 30, 30, 32/33 and 44/50 days
	" 310	Reedbuck ex sheep	6 rats not infected	
	" 334	Volunteer 280		
	(on three different days)			
Group IV	Volunteer 300 (twice)	Sheep line	None of the 20 rats infected	—
	" 349	" "		
	" 350	" "		
	" 353	" "		
	" 356	" "		
	" 334 (twice)	Volunteer 280		

The rats were examined daily (fresh blood films) for the first 30 days, and then twice weekly for the next 30 days, before they were considered to be negative. However, these examinations could not always be rigidly adhered to; hence some of the incubation-periods can only be given within limits of several days. (An incubation-period of 44/50 days means that the examination on the 43rd day was negative, but that trypanosomes were found on the 50th day.)

The results of the inoculation of rats with the trypanosomes found early in the infections of the men could be divided into four groups. The two or three rats inoculated from each infection or relapse were taken together and placed in the appropriate group.

**Group I.** All the rats were infected, with incubation-periods of 4-8 days, which is normal for this strain of *T. rhodesiense*.



*Group II.* All the rats were infected, but the incubation-periods were prolonged—9–18 days.

*Group III.* Only some of the rats were infected. Those rats which were infected had incubation-periods of 8, 10, 14, 16, 21, 30, 30, 32/33 and 44/50 days.

*Group IV.* Not one of the rats was infected.

The details of these groups are shown in Table II. It will be seen that all the rats in group I were inoculated with blood from men who had been infected from the Thomson's gazelle or monkey lines. In group IV the rats had been inoculated from men infected from the sheep line or from volunteer 280. In groups II and III the men had been infected from the sheep line or from other volunteers who had been infected from volunteer 280.

It was considered that a study of the morphology of the inoculated trypanosomes might give a clue to these results. By means of a camera lucida a line was drawn down the centre of the image of the trypanosomes found in the thin film, and the length of the line was then measured with dividers set at an interval of  $1\mu$ . The trypanosomes were classified as short, intermediate and long forms, and as positively or negatively charged, as described by Fairbairn and Culwick (1946).

While the lengths of each of the six types, in well-established infections in rats, are always normally distributed, it has been found that there may be statistically significant fluctuations in their mean lengths from day to day. The length distribution and the range within which the means may vary have been found to be as follows :

Positively charged forms	Length distribution	Range of means
Short	$12\mu$ – $26\mu$	$18.451 \pm 0.10$ — $20.351 \pm 0.170$
Intermediate	$17\mu$ – $30\mu$	$22.806 \pm 0.153$ — $24.313 \pm 0.172$
Long	$20\mu$ – $39\mu$	$28.560 \pm 0.214$ — $29.880 \pm 0.215$
Negatively charged forms	Length distribution	Range of means
Short	$12\mu$ – $24\mu$	$16.722 \pm 0.179$ — $18.323 \pm 0.181$
Intermediate	$16\mu$ – $31\mu$	$22.275 \pm 0.189$ — $22.385 \pm 0.167$
Long	$20\mu$ – $39\mu$	$28.387 \pm 0.172$ — $28.673 \pm 0.133$

The types and the length distributions of the trypanosomes found in the four groups are shown in Table III. Owing to the scarcity of the negatively charged forms, the means and the length distributions could only be worked out for the positively charged forms.

In the accompanying fig. the length distribution of the *positively* charged trypanosomes in each group are shown graphically.

In group I the trypanosomes present consisted only of the long and intermediate forms, with the exception of a single positively charged short form. There was a marked preponderance of the positively charged long forms. The lengths of the positively charged long and intermediate forms were normally distributed, and their mean lengths were within the range of means of similar types in well-established infections in rats. Of the long forms, eight (29.3 per cent.) of the negatively charged and 153 (38.3 per cent.) of the positively charged type were in a state of division. The lengths of the latter were normally distributed, with a mean of  $29.33\mu \pm 0.200$ .

In group II all six types of trypanosomes were present, and the positively charged

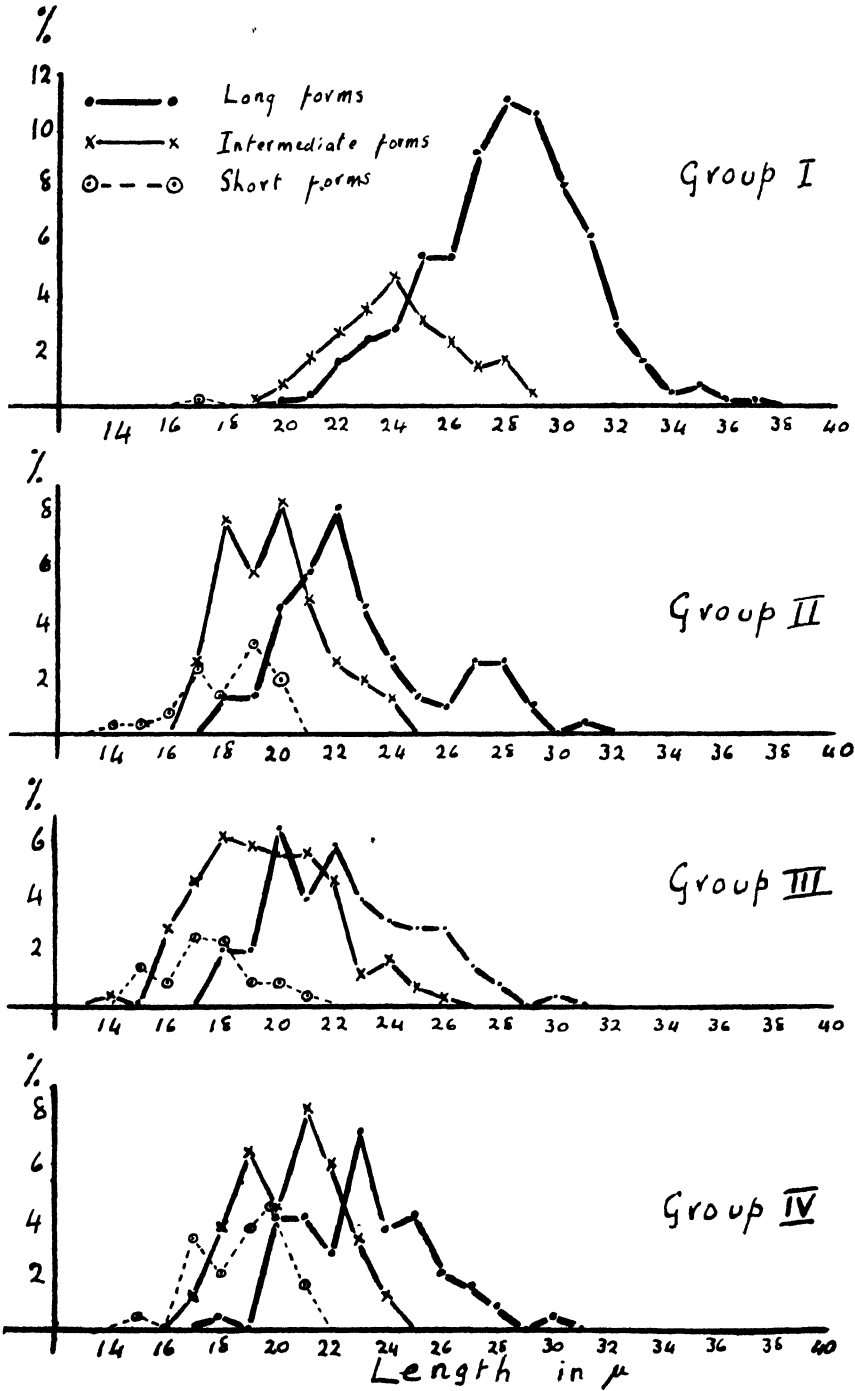


FIG. The length distribution of the positively charged trypanosomes in each group.

TABLE  
The length distributions and means of the

	Type of trypanosomes	Length in													
		12	13	14	15	16	17	18	19	20	21	22	23	24	25
Group I	Long negative											1	1	1	—
	" positive								1	2	9	13	16	32	
	Intermediate negative								1	1	4	2	4	1	3
	" positive								1	4	10	15	20	27	18
	Short "						1								
Group II	Long negative									1	6	5	5	2	5
	" positive							4	4	14	18	25	14	8	4
	Intermediate negative						2	1	—	4	1	2	—	—	1
	" positive						8	24	18	26	15	8	6	4	
	Short negative				1	1	2	3	—	2					
Group III	" positive			1	1	2	7	4	10	6					
	Long negative								2	4	3	3	4	2	1
	" positive							7	7	23	14	21	14	11	10
	Intermediate negative					2	4	3	6	2	3	6	1	2	1
	" positive			1	—	10	16	22	21	20	20	16	4	6	2
Group IV	Short negative	1	—	1	—	1	—	2	—	2					
	" positive					5	3	9	8	3	3	1			
	Long negative							1	—	2	1	2	1	1	—
	" positive							1	—	10	10	7	18	9	10
	Intermediate negative						4	3	4	7	4	2	1	4	1
Group IV	" positive						3	9	16	11	20	15	8	3	
	Short negative				1	—	—	1	—	—	2	1			
	" positive				1	—	8	5	9	11	4				

long and intermediate forms were now equal in numbers. The lengths of these two forms were not normally distributed, and their mean lengths were markedly shortened. Of the long forms, three (9.7 per cent.) of the negatively charged and five (4.4 per cent.) of the positively charged types were in division.

In group III all six types were present, and the positively charged long and intermediate forms were again in equal numbers. The lengths of these two forms were now normally distributed, but their mean lengths were markedly shortened. Of the long

## III

## trypanosomes from early infections in man

microns ( $\mu$ )														Mean length	Distribution (from $g_1$ and $g_2$ )
26	27	28	29	30	31	32	33	34	35	36	37				
2	7	6	3	4	1	1	1						28		
32	53	64	61	46	35	17	9	3	4	1	1		399	27.952 $\pm$ 0.131	Normal
2	1	—	1										20		
13	8	10	3										129	24.171 $\pm$ 0.175	Normal
													1		
2	1	3	1										31		
3	8	8	3	—	1								114	22.860 $\pm$ 0.270	Asymmetrical
1													12		
													109	19.771 $\pm$ 0.173	Asymmetrical
													9		
													31		
—	2												21		
10	5	3	—	1									126	22.540 $\pm$ 0.228	Normal
													30		
1													139	19.604 $\pm$ 0.195	Normal
													7		
													32		
1	1												10		
5	4	2	—	1	1								78	23.295 $\pm$ 0.284	Asymmetrical
													30		
													85	20.506 $\pm$ 0.190	Normal
													5		
													38		

forms, one (5.0 per cent.) of the negatively charged and eight (6.3 per cent.) of the positively charged type were in division.

In group IV all six types were present. The mean lengths of the long and intermediate positively charged forms, present in equal numbers, were again markedly shortened, but only in the latter form were the lengths normally distributed. None of the negatively charged long forms, but 20 (25.6 per cent.) of the positively charged ones, were dividing.

## DISCUSSION

In groups II, III and IV, not only were the mean lengths of the long and intermediate forms markedly shortened, but their appearance was also altered. Both forms were stouter than normal and the length of the free flagellum was reduced, so that it was often difficult to distinguish between the long and intermediate and between the intermediate and short forms. The appearance of the trypanosomes was so characteristic that one was able to forecast that the results of their inoculation into rats would be atypical. The whole picture suggested that these trypanosomes were 'immature.'

Fairbairn and Culwick (1946) have postulated that the intermediate and short forms are produced by syngamy (i.e., conjugation) of the heterozygous long form, and that such conjugation depends upon the presence of both the positively and the negatively charged types. The absolute increase in numbers of trypanosomes, i.e., multiplication, is due to division of the long forms alone.

On this hypothesis infection is produced by the inoculation of the long forms. In group I the long forms had normal length distributions and normal mean lengths, and all the rats were infected with normal short incubation-periods.

In group II the mean lengths of both types of the long trypanosomes were markedly reduced, but there was still a proportion of the positively and negatively charged types whose lengths exceeded the normal means.

Taking the normal mean length of the positively charged long trypanosome as  $29\mu$  and that of the negatively charged ones as  $28\mu$ , it is suggested that infection or non-infection of rats and the length of the incubation-period in the infected rats depend upon the *absolute numbers* of the long forms of  $29\mu$  and  $28\mu$  and over which are inoculated. In other words, it is only the long forms, whose lengths are greater than the normal means, which are 'mature' and capable of entering into conjugation and producing an infection.

This argument is not applicable to syringe-passaged strains. Fairbairn and Culwick (1947) have shown that a strain of *T. rhodesiense* from this laboratory, which had been maintained by syringe passage in mice in London since 1937, had altered significantly. It is now monomorphic, consisting only of long forms, and the mean length of the positively charged type has been reduced by over  $5\mu$  to  $23.29\mu$ . The inoculation of a single trypanosome of this syringe-passaged strain produces an infection in mice. The ability of such a long form to multiply and infect the host is probably due to genetic segregation having taken place, as it is certainly unable to enter into conjugation and produce intermediate and short forms.

Wenyon (1926) recorded that the inoculation into rats of fairly large quantities of blood known to contain *T. gambiense* had not infrequently failed to give rise to any recognizable infection. *T. gambiense* inoculated from man into rats may fail to infect them, and those that are infected have a chronic type of infection. Monkeys, however, are readily infected with *T. gambiense* from man, and after the strain has been passaged through a monkey it becomes more virulent for the smaller animals.

It would be of interest to measure the trypanosomes taken from man and monkeys infected with *T. gambiense*, to see what types are present and their length distributions, and to compare them with the results of the present investigation.

## SUMMARY

It was found that rats inoculated with human blood containing trypanosomes (*T. rhodesiense*) did not necessarily become infected.

The blood from the arm-reaction or the venus blood was inoculated into rats as soon as trypanosomes had been found in stained thick films, i.e., at the earliest possible moment in the infection of the men.

The morphology of the inoculated trypanosomes was studied in thin films.

The effects of inoculation in rats could be divided into four distinct groups, depending on the incubation-periods and on whether or not the rats became infected.

The length distributions and the mean lengths of the trypanosomes in each group is recorded.

The hypothesis is advanced that it is only the positively and negatively charged long forms, whose lengths are greater than the means of these types, which are infective to rats.

It is suggested that this work should be repeated in men and monkeys infected with *T. gambiense*, to see if comparable results are obtained.

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# THE EXCRETION OF STILBAMIDINE AND SOME RELATED COMPOUNDS IN EXPERIMENTAL ANIMALS

BY

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The excretion of 4:4'-diamidino stilbene (stilbamidine) has been studied qualitatively in mice by Hawking and Smiles (1941). As yet it has been studied quantitatively only in the human. Thus, Henry and Grindley (1942) found that in two human subjects less than 10 per cent. of a single intravenous dose could be detected in the urine in a fluorescent form within the next three days; while Kirk and Henry (1944) reported that, during a course in which nine intravenous injections of 50 mgm. each were given on alternate days, excretion of the compound in a fluorescent form, though at first small, increased until finally about 80 per cent. of each dose was eliminated, the major part during the following 24 hours. Their results also demonstrated the existence of a saturation phenomenon, which in their experiments appeared after five doses of the course. In view of these findings, there appeared to be sufficient evidence to warrant more detailed investigations with laboratory animals, a preliminary account of which has been given by Wien (1946).

The present studies were therefore undertaken to examine the urinary excretion after single and repeated injections of stilbamidine and some closely related compounds in rabbits and rats. While interest was centred on stilbamidine, experiments were also carried out with the substituted compounds 4:4'-diamidino-2-hydroxystilbene (hydroxystilbamidine), 4:4'-diamidino-2-aminostilbene (aminostilbamidine), and 4:4'-diamidino-2-iodostilbene (iodostilbamidine).

## METHODS OF DETERMINATION

In the initial experiments stilbamidine was determined only by a modification of the fluorimetric method of Henry and Grindley (1942). Later, however, when the adaptation, described below, of the colorimetric method of Fuller (1944, 1945) had been evolved, both methods were used. The other compounds studied were determined in the following manner: aminostilbamidine both by the fluorimetric method and by Hall's (1942) modification of the diazotization method of Bratton and Marshall (1939) for the determination of sulphonamides; iodostilbamidine, being non-fluorescent, by the colorimetric method described below; and hydroxystilbamidine, which is neither fluorescent nor reacts readily with glyoxal, by the yellow colour of the base itself in the butanol extract. The sensitive method of Brodie and Udenfriend (1945) for the determination of organic bases was not applicable, though stilbamidine does form a compound with methyl orange.

### *(a) Fluorimetric Method*

The method of Henry and Grindley was modified in the following manner. By substituting a moistened small disc of filter-paper (Whatman's no. 1, 18 mm. diameter)\*

\* This is the smallest size available commercially.

for the larger sheet used by Henry and Grindley, an even diffuse fluorescence was produced over the whole paper. This was found to be easier to compare than the circles of fluorescence given by the original method. It was found necessary to neutralize the urine. Since these experiments were carried out, improvements in their original method have been reported by Henry and Grindley (1945), which allow of much greater accuracy.

### (b) *Colorimetric Method*

Two methods for the colorimetric determination of aromatic amidines in biological fluids by means of a reaction with glyoxal have been reported. The method of Devine (1944) was found to be applicable to stilbamidine only in pure solution, and not in the presence of urine or plasma. The method described by Fuller (1944, 1945), however, was found to be much more sensitive and to be applicable not only to stilbamidine but to many other aromatic mono- and diamidines. The optimal conditions defined by Fuller were confirmed for this reaction, and it was also found that, while aromatic monoamidines require approximately two molecules of glyoxal for maximum colour production, aromatic diamidines require similarly four molecules of glyoxal. However, the reaction was partially or wholly inhibited in animal urines. Similarly, it was inhibited by aqueous solutions of sodium chloride or urea. In attempting to overcome this difficulty, extraction with immiscible solvents was investigated. While amidine bases themselves are almost insoluble in organic solvents, the small amounts encountered in practice are easily soluble in certain water-saturated solvents, such as butyl, amyl, isobutyl and isoamyl alcohols, but are not soluble in acetone, ether, benzene, petroleum ether or carbon tetrachloride. It was thus found not only that the bases could be extracted from alkaline solution, but also that the colour reaction could proceed in the solvent. This eliminated the precipitation of the coloured complex frequently encountered when the reaction was carried out in aqueous solution. Therefore in the following method butyl alcohol was used for the extraction of the amidine, which was then determined by a colorimetric method dependent on the reaction with glyoxal.

#### *Reagents*

n-butyl alcohol.

Borate buffer: 4 gm. boric acid dissolved in hot distilled water titrated to pH 9.0 with NaOH, and made up to 100 ml. with distilled water.

Glyoxal reagent: glyoxal sodium bisulphite (B.D.H.) 0.625 per cent. in distilled water.

Methylated spirit.

Glacial acetic acid.

*Method.* Ten ml. of urine, containing not more than 1 mgm. of amidine (as base), is made alkaline to litmus and shaken vigorously with 15 ml. butanol in a separating funnel for five minutes. After centrifuging, 7.5 ml. of the butanol extract is taken off and washed with 3 ml. borate buffer in a separating funnel. After centrifuging, a 5 ml. aliquot of the butanol extract is pipetted into a test-tube, 0.1 ml. of glyoxal reagent, 0.05 ml. borate buffer and 1.0 ml. methylated spirit are added, the contents are well mixed, and the tube is placed in a boiling water bath for 10 minutes. The volume is then made up to 6 ml. with a 5 : 1 mixture of butanol-methylated spirit, 1.0 ml. glacial acetic acid is added, and the colour is measured in a photoelectric absorptiometer, using filters having a maximum transmission at 530 m $\mu$  (Ilford no. 405).

This method is applicable to solutions of stilbamidine, 4 : 4'-diamidino diphenyl ether (phenamidine), 4 : 4'-diamidino diphenoxy propane (propamidine), 4 : 4'-diamidino



diphenoxy pentane (pentamidine), and to some derivatives of these compounds. The colours given vary from pink to magenta, but all give strong absorption at 530 m $\mu$ . The amount of solvent necessary for the extraction of these compounds from 10 ml. urine varies: in the case of stilbamidine 15 ml. was necessary, while 10 ml. sufficed for phenamidine, propamidine and pentamidine. The smallest amount of amidine detectable by this method is about 0.1 mgm. (as base) in 10 ml. urine, with an error not greater than 0.1 mgm. Recovery from urine is good in the case of propamidine, but with stilbamidine there is a constant loss of about 0.1 mgm over the range 0.1 to 1.0 mgm. The method gives reproducible results, however, when allowance is made for incomplete recovery by the use of calibration curves.

It should be noted that neither the above method nor the method of Fuller (1944, 1945) is applicable to the determination of stilbamidine in plasma or serum, as neither is sufficiently sensitive to detect the small concentrations present after injection of the drug.

### EXPERIMENTAL

In all experiments calculations of dosage and excretion are stated in terms of base, though solutions were made up for dosage from the di-isethionate or dihydrochloride salt. All animals were kept in metal metabolism cages. The rabbits were fed on a diet of bran oats and dried sugar-beet pulp, moistened with water, and the rats on a standard rat diet, as used in these laboratories.

#### *The Excretion of a Single Dose of Stilbamidine*

Two rabbits were injected intravenously with 20 mgm. of base (10 mgm./kgm.) as the di-isethionate, and the stilbamidine excreted in the urine was determined by the fluorimetric method. From the results given in Table I it will be seen that, although only a small percentage of the administered compound was excreted, the major part of this was recovered within the first 24 hours, and that small amounts were being excreted up to five days after injection.

TABLE I  
The urinary excretion of a single intravenous injection of 10 mgm./kgm. of stilbamidine in rabbits (fluorimetric method)

Time after injection	Milligrams of drug excreted (as base)	
	Rabbit A	Rabbit B
24 hours	0.11	0.05
48 "	0.07	0.02
96 "	0.03	0.01
120 "	0.01	0.00
Total excreted ... ..	0.22 mgm.	0.08 mgm.
Percentage of dose excreted ...	1.1	0.4

#### *The Excretion of Stilbamidine After Repeated Injections*

Two rabbits were injected intravenously with 5 mgm./kgm. on five alternate days.

This was followed after 48 hours by a final dose of 10 mgm./kgm. Urine was collected at 24-hourly intervals, pooled and the stilbamidine present determined by the fluorimetric method. Table II shows the amount excreted in this experiment.

TABLE II

The urinary excretion of stilbamidine by two rabbits after repeated intravenous injections (fluorimetric method)

Days	Dose administered	Milligrams excreted	Percentage of each dose excreted	Percentage of total dose excreted
1	38 mgm.	0.59	1.55	1.55
2	—	0.285	0.75	2.3
3	38 mgm.	0.07	0.18	1.24
4	—	0.27	0.71	1.6
5	38 mgm. (5 mgm./kgm.)	0.66	1.73	1.64
6	—	0.46	1.21	2.05
7	38 mgm.	0.92	2.42	2.14
8	—	0.90	2.37	2.74
9	38 mgm.	0.56	1.47	2.48
10	—	0.49	1.29	2.74
11	76 mgm. (10 mgm./kgm.)	0.295	0.78	2.90
12	—	0.68	0.89	2.32
13	—	0.09	0.12	2.36
Total	266 mgm.	6.27 mgm.	—	2.36 per cent.

It will be seen that after each dose a small but relatively constant amount was excreted, though after the final dose of 10 mgm./kgm. no increase was detected and in all only 2.4 per cent. of the whole amount injected was excreted in a fluorescent form.

Rats can tolerate larger amounts of stilbamidine than rabbits, and it was possible to carry out longer experiments with a larger number of daily injections with these animals. It was necessary, however, to give the compound subcutaneously, owing to the difficulty of giving a large number of intravenous injections into the caudal veins of the rat.

The animals were kept in metal metabolism cages, except for a period of one hour each day when they were taken out for feeding. This measure was necessary to eliminate the contamination of the cages with foodstuffs on which stilbamidine is strongly adsorbed. It was difficult to prevent the occasional adsorption of the drug on to faeces in the metabolism cages, and this source of error was not entirely obviated.

Two experiments were carried out in which the excretion was determined after daily subcutaneous injections of stilbamidine. In the first experiment 15 daily doses, each of 1.0 and 2.0 mgm./kgm., were given to two groups of four rats in each group, and in the second experiment 23 doses of 5.0 and 10.0 mgm./kgm. were given to two groups of nine rats in each group. Urines were collected daily and determinations were carried out by the fluorimetric method. The results obtained from both these experiments are given in Table III.

Three interesting observations were apparent in these results. Firstly, the animals receiving small doses did not excrete any detectable amount in the urine until five doses had been given. Secondly, in all four groups excretion was at first fairly consistently low, but later a sudden rise occurred after about 10 doses had been given, irrespective of the size of the dose, and remained at a much higher level until the end of the experiment. Thirdly, a more interesting observation was evident in that proportionately very much less of the compound was excreted by the animals receiving large doses than by those

TABLE III

The urinary excretion of stilbamidine in rats after daily subcutaneous injections of 1.0, 2.0, 5.0 and 10 mgm./kgm.

Dose	First experiment				Second experiment			
	1.0 mgm./kgm.		2.0 mgm./kgm.		5.0 mgm./kgm.		10.0 mgm./kgm.	
Days	A	B	A	B	A	B	A	B
1	—	—	—	—	1.7	1.7	0.8	0.8
2	—	—	—	—	2.4	2.1	1.8	1.3
3	—	—	—	—	0.6	1.5	0.4	0.9
4	—	—	—	—	1.2*	2.7	1.4*	2.0
5	7.2*	1.4	7.3*	1.3	0.8	1.3	0.8	1.0
6	6.1	2.2	14.0	3.3	0.2	1.1	0.7	0.9
7	4.8	2.6	13.0	4.5	0.8	1.1	0.4	0.9
8	7.2	3.2	12.0	5.4	0.7	1.0	0.4	0.8
9	25.0	5.7	23.0	7.2	3.3	1.3	0.2	0.7
10	15.0*	6.6	13.0*	7.7	1.1	1.3	0.6	0.7
11	24.0	8.1	5.0	7.5	5.5	1.7	2.0	0.8
12	27.0	9.8	35.0	9.8	2.5	1.8	2.2	0.9
13	22.0	10.7	6.3	9.6	4.9*	3.8	4.5*	2.1
14	13.0	10.9	0.0	9.0	1.9	2.0	1.3	1.2
15	4.8	10.4	0.0	8.4	7.5	2.2	3.1	1.2
16					6.1	2.3	3.0	1.3
17					4.0	2.4	3.5	1.4
18					2.1	2.4	2.0	1.4
19					1.5*	4.5	2.3*	1.8
20					2.4	2.3	0.8	1.4
21					2.3	2.3	1.3	1.4
22					2.1	2.3	2.2	1.5
23					3.6	2.4	1.3	1.5
30						3.0		1.8

A = Percentage of the daily dose excreted in the following 24 hours.

B = Total amount excreted as a percentage of the total dose given.

\* Excretion after 48 hours.

receiving small doses, when the reverse was expected. This phenomenon has been confirmed in later experiments. The actual amount of stilbamidine excreted by each group is shown in Table IV as milligrams excreted per 100 gm. body-weight, and it will be seen that there is a marked similarity in the amounts excreted by animals receiving different doses.

TABLE IV

The amounts of stilbamidine excreted by rats receiving 1.0, 2.0, 5.0 and 10.0 mgm./kgm./diem subcutaneously, as determined by the fluorimetric method (in milligrams per 100 gm. body-weight)

Dose ... ..	1.0 mgm./kgm.	2.0 mgm./kgm.	5.0 mgm./kgm.	10.0 mgm./kgm.
After 5 doses ...	0.007	0.014	0.039	0.048
" 10 " ...	0.064	0.156	0.079	0.078
" 15 " ...	0.142	0.266	0.202	0.244
Total amount injected mgm./100 gm. ...	1.5	3.0	7.5	15.0

A further experiment was carried out in which a daily dose of 0.5 mgm./kgm. was given, but the amount of stilbamidine in the urine was too small to be determined with any accuracy.

When the colorimetric method became available, experiments were carried out to investigate and compare the excretion of stilbamidine, iodostilbamidine and hydroxystilbamidine by both this and the fluorimetric method. Stilbamidine was determined by both methods, and iodostilbamidine and hydroxystilbamidine, being non-fluorescent, by the colorimetric methods described above.

These three compounds were administered subcutaneously in doses of 1.0 mgm./kgm./diem and 10 mgm./kgm./diem to groups of three rats, a total of 15 doses being given. Determinations were carried out on 24-hourly samples of urine. The figures obtained for the excretion of these compounds showed that, as had been noticed in an earlier experiment with stilbamidine, the initial delay in excretion followed by a marked rise was evident with each of these three substances, with stilbamidine after nine doses of 1 mgm./kgm./diem, with iodostilbamidine after four doses of 10 mgm./kgm./diem, and with hydroxystilbamidine after four doses of 1 mgm./kgm./diem. The same phenomenon had been observed in an earlier experiment with aminostilbamidine, in which it was administered subcutaneously in doses of 1.0 and 2.0 mgm./kgm./diem, where the sudden rise in excretion occurred after four doses of 1.0 and 2.0 mgm./kgm./diem had been given. This latter compound was determined by the fluorimetric method and by a diazotization method essentially similar to that used for the determination of sulphonamides. This effect was more marked in the determinations by fluorimetric than by colorimetric methods, but was not evident in all cases. As was found in previous experiments, proportionately very much less was excreted by animals receiving a high dose of 10 mgm./kgm./diem than by those receiving only 1 mgm./kgm./diem. This was shown with all the compounds and by both fluorimetric and colorimetric methods of determination. It was also shown in the case of aminostilbamidine, where the animals receiving 2 mgm./kgm./diem excreted proportionately less than those receiving 1 mgm./kgm./diem. Iodostilbamidine and aminostilbamidine were excreted in approximately the same amounts as was stilbamidine, where dosage was comparable, but less hydroxystilbamidine was excreted.

The interesting point disclosed by the use of the colorimetric method for the determination of stilbamidine was that the results obtained showed a urinary excretion about 20 times greater than was shown by the fluorimetric method. In the case of aminostilbamidine more of the compound was detected in the urine by the diazotization than by the fluorimetric method. It is not thought possible that experimental error could account for this large difference, which was consistent, and, as it was shown by Fuller (1945) that the glyoxal reaction is specific for amidine groups, it is concluded that a large proportion of the injected stilbamidine (about half a daily dose of 1 mgm./kgm.) was excreted in this experiment in the form of a non-fluorescent amidine.

An attempt was made to isolate and identify this excretory end-product. Purification was carried out by adsorption chromatography on alumina at pH 7.0 of a water-saturated butanol extract of the urine, followed by elution with acidified methylated spirit and evaporation to dryness 'in vacuo.' Considerable losses occurred, however, of the small amount present (initially 30 mgm.), and none of the compound was isolated.

## DISCUSSION

Storage in the body of injected compounds is a well-known occurrence, and the results of these experiments indicate that stilbamidine and those of its derivatives studied

here undergo a similar process, though it is not a simple case of storage, as indicated by the apparent saturation occurring after different amounts had been given.

The curious phenomenon disclosed in all these experiments, of diminishing excretion with increasing amounts administered, is of considerable interest, and some analogy may be found in the experiments of Bray, Neale and Thorpe (1946), who obtained similar results while studying the metabolism of sulphapyridine. Considerable variation in day-to-day excretion tends to discount a constant or maximal rate of excretion by the kidneys, and it seems that some metabolic or adsorptive process destroys amounts in excess of a threshold concentration in the body.

Some suggestions can be made, however, as to the nature of the excretory end-products on consideration of their loss of fluorescence. The fluorescence of stilbamidine is due to the conjugated structure of the molecule with its alternating double bonds, and it can be destroyed by saturating these bonds. As most of the amidine groups were intact in the excreted end-product, there appear to be at least three possible explanations for the loss of fluorescence.

The first is that the central double bond becomes saturated. It has been shown by Fulton (1943) that the addition of the elements of water occurs at this double bond when aqueous solutions of stilbamidine are exposed to light, though no other mild chemical treatment would produce a similar change. Further evidence for this was put forward by Barber *et al.* (1943). It has also been suggested by Henry (1943) that dimerization can occur.

The second possibility arose out of an investigation of the non-fluorescence of certain derivatives of stilbamidine, such as hydroxystilbamidine and iodostilbamidine, while other derivatives remain fluorescent or, as in the case of aminostilbamidine, are more intensely fluorescent than stilbamidine itself. It is interesting to note that, as shown by Williams (1928), the dipole moments of  $-\text{Br}$ ,  $-\text{Cl}$ ,  $-\text{I}$ ,  $-\text{NO}_2$ ,  $-\text{CHO}$ ,  $-\text{OH}$ ,  $-\text{OCH}_3$ , and  $-\text{COOH}$  groups on a benzene nucleus are negative in varying degrees, while those of  $-\text{NH}_2$  and  $-\text{CH}_3$  are positive. It is probable that the negative dipole moments of the hydroxyl group in hydroxystilbamidine and of the iodine atom in iodostilbamidine alter the electronic energy-levels in the nucleus in such a way as to destroy the possibility of fluorescence. This being so, it is then possible that the non-fluorescent metabolite of stilbamidine may have a substituted group on one of the benzene nuclei, of negative dipole moment.

Fission—reductive or oxidative—into two monoamidine residues is also possible, since the estimation procedure determines total amidine groups.

## SUMMARY

1. Fluorimetric and colorimetric methods for the determination of aromatic diamidines have been adapted for use with animal urines.

2. The urinary excretion of 4:4'-diamidino stilbene (stilbamidine), 4:4'-diamidino-2-aminostilbene, 4:4'-diamidino-2-iodostilbene and 4:4'-diamidino-2-hydroxystilbene has been studied in experimental animals after single and repeated injections.

3. It has been shown that storage phenomena are evident for all these compounds on repeated dosage and that excretion rises after the saturation point is reached. In the case of stilbamidine, saturation is reached no faster with large doses than with small doses.

4. Much larger proportions of the compounds were excreted after repeated administration of small doses (1 mgm./kgm./diem) than after large doses (10 mgm./kgm./diem).

5. It has been shown that small amounts of stilbamidine were excreted in a fluorescent form, but that much larger amounts of this and of the other compounds studied were excreted in a non-fluorescent form.

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NOTES ON THE TYPE-SPECIMENS OF AFRICAN  
TABANIDAE (DIPTERA) DESCRIBED BY  
MR. H. F. CARTER (1912, 1915)

BY

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In two papers Mr. H. F. Carter (1912, 1915) described 10 new species and one new variety of Tabanidae from West and South Africa, most of which have remained little known. Through the courtesy of Professor R. M. Gordon and Dr. D. S. Bertram I have been allowed to borrow the types of these species for study and comparison with material in the British Museum. I wish to express my gratitude for the opportunity of seeing these types, which are deposited in the Liverpool School of Tropical Medicine.

Carter's papers give very full and accurate descriptions and excellently clear figures. The present paper is in no sense an attempt to redescribe Carter's species, but is a record of a few points which come to light in comparing the type-specimens with later material, and in particular with Bequaert's (1930) key to the Tabanidae of the Belgian Congo, which is the best existing key to any Ethiopian species.

Measurements of the frons were made with a squared eyepiece, and the figures quoted are in units of the eyepiece and not in millimetres. The order is as follows: breadth of frons at vertex; breadth at antennal angles; length. 'Ratio' is length divided by breadth at vertex, 'Callus' (*Haematopota*) is the distance from the antennal angles to the highest point of the callus, in the same units (see fig.). The conversion factor for this eyepiece is 28 units equal 1 mm.

***Tabanus nagamiensis* Carter (1912, p.435)**

Frons 14 : 10 : 38	Ratio 2·7
„ 17 : 13 : 46	„ 2·7

I can add little to Carter's statement that this species resembles *diversus* Ric. in the form of the frontal callus, but is clearly distinct. The abdominal pattern is made up of three rows of triangles, the outer series being blunter and more prominent. There is no suggestion of a short median stripe as in *diversus* and *lufirensis* Beq., the median triangle on the second segment being as prominent as the rest.

In the British Museum are two of the co-types, collected by Dr. R. W. James, and other specimens from Rhodesia, Chirinda Forest, 3,800 ft., October 12th-15th, 1911 (Swynnerton); Belgian Congo, Katanga district, Kandelungu, September 28th, 1925 (Schwetz); Belgian Congo, Elisabethville, October 7th, 1926 (M. Bequaert); and Northern Rhodesia, near Muebe, Luena district, September 4th, 1904 (Harger).

Carter remarks that in several specimens the integument is distinctly dull orange-brown at the sides and along the apical margins of the abdominal segments. The British Museum has a short series from Pretoria and Zululand (G. A. H. Bedford) which differ from the typical form in this way.

***Tabanus fulvicapillus* Carter (1912, p.437)**

Frons 9 : 7 : 29      Ratio 3:2

" 8 : 6 : 27      " 3:4

As pointed out by Carter, this big yellow-and-black species is clearly Palaearctic in affinity. Carter compared it—rather strangely—with *trigonus* Coq., a Japanese species, and gave contrasting figures of the frontal callus in the two species. In *trigonus* the bulbous part of the callus is rounded in outline and widest in the middle. In *fulvicapillus* it is flask-shaped and widest at its lower margin, where it is cut off bluntly with a toothed edge, as in *T. bovinus* L. and *T. sudeticus* Zell. Indeed, *fulvicapillus* is very like *sudeticus*, except for its more pointed abdomen and conspicuous yellow hairs on the fore coxae and first abdominal segment. It seems much more likely to be an isolated offshoot from the European *sudeticus* group than any close relative of the Japanese *trigonus*.

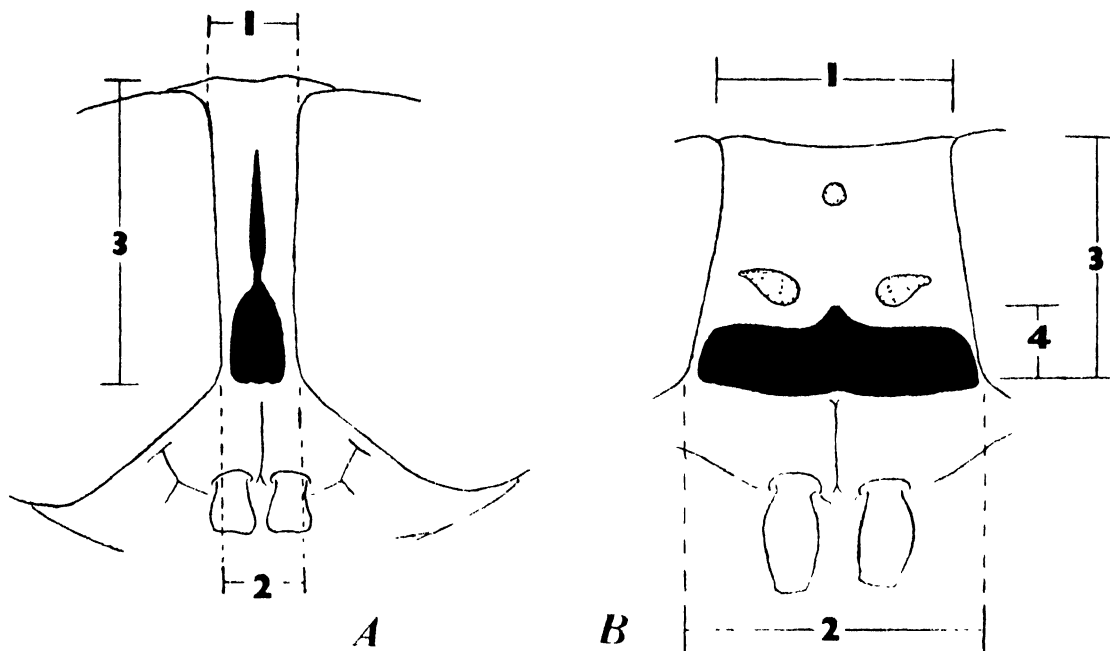


FIG. Heads of *Tabanus* (A) and *Haematopota* (B) (schematic), to illustrate frons measurements. 1.—Breadth at vertex; 2.—Breadth at antennal angles; 3.—Length; 4.—'Callus' (in *Haematopota* only).

Bequaert (1930) knew this species only from the description. He keys it out easily, early in his group G, but in the text (p.931) he compares it with *roubaudi* Surc. There is, in fact, no close relationship between *fulvicapillus* and the *roubaudi-wellmanni* group of small, clearly marked, dark species.

***Tabanus donaldsoni* Carter (1912, p.439)**

Frons 7 : 5 : 26      Ratio 3:7

This big bright-orange species is very well described by Carter. In Bequaert's key it runs to couplet 23 (p.909) and may be separated by inserting a new couplet between 22 and 23.



- 22.1. Wings relatively long, equal to full length of the body, noticeably infuscated along veins, especially on radial fork. Larger species (17 mm. upwards) with bright orange antennae and abdomen. Frons ratio 3.7 *donaldsoni* Carter  
 Wings not relatively long nor markedly infuscated. If body longer than 17 mm., then of darker colour, antennae dark brown to black, frons ratio between 6 and 7 ... .. 23

Type locality Ashanti, Bromassie; also recorded from Southern Nigeria, Calabar. In the British Museum there are specimens from 'S. Nigeria' (H. A. Foy) and the Gold Coast, Northern Territories, Bandewa, May 26th, 1913 (J. J. Simpson).

***Tabanus triquetroratus* Carter (1915, p.173)**

Frons 13 : 7 : 58      Ratio 4.5

This species has been confused with *argenteus* Surc., with which Carter compared it, but it is easily distinguished by having a large median triangle on the second abdominal tergite. At couplet 36 of Bequaert's key this leads, not to 37 and the *wellmanni* group, but via 42 to couplet 58, where a new couplet may be inserted.

- 57.1. Frons long, narrow, sharply tapered, nearly twice as wide at vertex as at antennae. Callus square, box-like, with thin extension ... .. *triquetroratus* Carter  
 Frons wider, not so sharply tapered, or with a different form of callus ... 58

The form of the callus and the thoracic ornamentation show *triquetroratus* to be related to *argenteus* and *wellmanni*, but the abdominal pattern sets it apart.

The British Museum has specimens from Sierra Leone, Njala, at light, March 28th, 1935 (E. Hargreaves), and the Imperial Institute of Entomology from Sierra Leone, Masamberi, February 15th, 1913 (J. Y. Wood).

***Tabanus (Ochrops) fuscipes* Ric. var. *oculipilus* Carter (1915, p.175)**

Frons 20 : 17 : 45      Ratio 2.3

The length of the ocular hairs in *fuscipes* seems to be variable, and, although specimens with paler legs can be found, it is doubtful whether this variety is sufficiently distinct to justify a name. The antennae of Ricardo's type are of the shorter form which Carter figures for his variety.

**HAEMATOPOTA**

A great many African species of *Haematopota* are still undescribed, and major groups within the genus are difficult to define with precision. Bequaert's key to the Congo species is the only practical key yet published, but the 33 species included are certainly less than one-fifth of those existing in the Ethiopian Region. I have tried to indicate how Carter's species run down in this key, but at best this process can show only approximate relationships.

***Haematopota transvaalensis* Carter (1915, p.176)**

Frons 33 : 37 : 35      Ratio 1.1      Callus 12  
 .. 30 : 33 : 35      .. 1.1      .. 12

This species runs down to *stimulans* Austen, from which it differs in a number of comparative characters: more *Tabanus*-like, heavily patterned abdomen; darker, more

vividly ringed legs ; more robust antennae ; callus more produced in middle line ; bigger frontal spots ; pale areas of wing less developed. As positive marks of identification are the pale markings in the discal cell, which form an X-shape, and the pair of admedian thoracic stripes, each of which ends in a conspicuous white spot.

***Haematopota theobaldi* Carter (1915, p.179)**

Frons 30 : 37 : 27	Ratio 0.9	Callus 8
" 27 : 33 : 25	" 0.9	" 8

In this species the first antennal segment is swollen and slightly hollowed on the upper surface, but without a distinct preapical cleft. In Bequaert's key it thus runs to couplet 31, whence the key may be expanded to read :

- |       |   |                         |
|-------|---|-------------------------|
| 31.   | Frons distinctly narrowed towards vertex ... ..   | 31.1                    |
|       | Frons nearly parallel-sided ... ..  | 33                      |
| 31.1. | Face uniformly grey throughout. Antennae black ... ..   | 32                      |
|       | Face with velvety-brown crescentic mark on each parafacial, and a reddish-brown spot under each antenna. First antennal segment shining mahogany-brown (not black, as given by Carter) ; third segment reddish, following segments black ... .. | <i>theobaldi</i> Carter |

In the British Museum are two females from the type locality, Onderstepoort, Transvaal (Bedford), and a series of both sexes from Weenen, Natal (Thomasset). The male is very similar to the female, differing chiefly in the shorter antennae, of which the first segment is swollen and shining, the third elongate oval. In the eyes the upper facets are enlarged and paler than the lower ones (dried specimen).

***Haematopota pinguecornis* Carter (1915, p.182)**

Carter's figure of this species shows no constriction near the tip of the first antennal segment, and—presumably on the strength of this—Bequaert includes it in his list of species with a broad disciform third segment and no constriction. The antennae of the type are broken, and obscured by adhesive, but one specimen in the British Museum shows a distinct furrow (the second specimen has no head).

On this basis the species runs to couplet 20 in Bequaert's key, and is separated from *vittata* as follows :

- |       |  |                            |
|-------|--|----------------------------|
| 20.1. | Larger species (11 mm.), more brownish. Frons 33 : 35 : 37 ; ratio 1.1 ; callus 8. Third antennal segment conical ... .. | <i>vittata</i> Loew        |
|       | Smaller species (7 mm.), very black. Frons 25 : 28 : 22 ; ratio 0.9 ; callus 4. Third antennal segment short oval ... .. | <i>pinguecornis</i> Carter |

In the British Museum there are two females : from Northern Nigeria, Ipana, September 10th, 1910 (Simpson) ; and from Northern Nigeria, Yola province, Pella, August 4th, 1909 (Dalziel).

***Haematopota angustifrons* Carter (1915, p.185)**

Frons 23 : 27 : 35	Ratio 1.5	Callus 10
" 21 : 26 : 32	" 1.5	" 9
" 21 : 26 : 35	" 1.7	" 10

This species is included in Bequaert's key and in the text, where it is stated that *H. pallidicornis* Edw. is 'extremely close.' The type of *pallidicornis* is in the British

Museum, and runs down to *angustifrons* in the key. At first sight *pallidicornis* seems to have the frons narrower at the vertex and the palpi more slender, but these are variable in the five specimens of the two species available, and it seems likely that the two are forms of one species. *H. angustifrons* is described from the Congo, and *pallidicornis* Edw. (1916, p.149) is from the Cross River, Southern Nigeria. Frons measurements of *pallidicornis* are 17 : 22 : 32 ; ratio 1.8 ; callus 10.

***Haematopota exiguicornuta* Carter (1915, p.188)**

***Haematopota corsoni* Carter (1915, p.190)**

Frons 19 : 22 : 18 (deformed)	Ratio 1	Callus 5
" 22 : 25 : 21	" 1	" 5

These two species, though distinct, are close to *brunnescens* Ric., differing from the latter and from each other chiefly in the proportions of the antennal segments and in the wing-markings. In certain lights, as pointed out by Carter, the hind tibiae of *corsoni* show two pale rings, and it should therefore be treated for the purposes of a key as having ringed tibiae. Bequaert meets the dilemma of these doubtfully marked species by making the expanded third antennal segment a second character. This is present in *corsoni*, which therefore goes to couplet 9 and so on to *brunnescens*, couplet 29.

The three species may be separated thus :

- |       |   |                             |
|-------|---|-----------------------------|
| 29.   | Frons rather narrow, etc. ....  | <i>rufula</i> Surc.         |
|       | Frons wider, not more than 1½ times as long as wide at vertex. Basal callosity short, occupying not more than one-quarter of frons, which has three velvety spots ....          | 29.1                        |
| 29.1. | Flagellar segments together twice as long as scape + pedicel ; third segment elongate oval, 1½ times as long as broad. Abdominal admedian spots very large and distinct ....    | <i>brunnescens</i> Ric.     |
|       | Flagellar segments together less than twice as long as scape + pedicel. Third segment disciform. Abdominal admedian spots not distinct ...                                      | 29.2                        |
| 29.2. | Rings of hind tibiae almost invisible. Wing-markings reduced to isolated spots. Third antennal segment about as long as the following segments together ....                    | <i>corsoni</i> Carter       |
|       | Rings of hind tibiae moderately distinct. Discal cell with three pale areas, wing-markings normal. Third antennal segment twice as long as the following segments together .... | <i>exiguicornuta</i> Carter |

A long series in the British Museum from the Gold Coast, Northern Territories (Beringer), is very close to *corsoni*, but is bigger and somewhat intermediate between *corsoni* and *brunnescens*. Three specimens from the Gold Coast, Northern Territories, Wa, have the antennae of *corsoni* but a different wing-marking. Evidently a number of species, or a number of forms of one species, centre around *H. brunnescens* Ric.

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# NOTES ON THE EARLY STAGES OF CERTAIN ETHIOPIAN MOSQUITOES, WITH SOME LOCALITY RECORDS FROM BRITISH WEST AFRICA

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## NOTES ON THE EARLY STAGES

### **Anopheles domicolus** Edwards

The unassociated larvae attributed to this species by Evans (1938) are now known to have belonged to some other species. The larva described by the present author as possibly that of *A. theileri* var. *brohieri* (Mattingly, 1944) has been shown to belong to *A. domicolus* by Mr. F. Y. Brown and Dr. G. A. Walton, who bred out identical larvae in Nigeria and Sierra Leone respectively. The descriptions which follow are based on two larval and three pupal pelts from Lokoja, Nigeria (F. Y. Brown), two whole larvae taken by M. D. Froud and the author at Kaduna, Nigeria, a pelt of the author's from Kintampo, Gold Coast, and a description and drawings received from Dr. G. A. Walton of 30 larvae collected and bred out by him from the Orugu River below Speke Hill near Freetown, Sierra Leone. The description and drawings agree closely with the remaining material, which is now in the British Museum.

**LARVA** (fig. 1). *Colour*. 'Dark green, speckled with black pigment, a closer concentration of dark pigment down the centre of the abdomen divides into two arms on the thorax extending from the centre of the posterior thoracic margin to the shoulders. A paler area on either side of the thorax contains a dense black spot. Sides of abdomen darker when seen from above' (Walton). Head brownish-yellow, with two transverse brown bands and a small brown triangle posteriorly. The anterior transverse band is apparently darker and more uniform in Sierra Leone specimens than in Gold Coast and Nigerian material, and the posterior triangle is connected to the posterior transverse band in the former but not in the latter. The edges of the posterior half of the epicranial suture are pigmented in all cases.

*Head. Clypeal Hairs*. *Inner* long, slender, simple, their bases widely separated; *outer* slender, 2-3 branched, about  $\frac{1}{2}$  the length of the inner; *posterior* slender, bifid, their tips reaching just beyond the bases of the inner. *Pre-clypeals* rather long and slender. *Mid-frontal hairs* with 6-12 branches, the two inner about twice as long as the four outer or more. *Post-frontal hairs* slender, simple, their tips not quite reaching the bases of the mid-frontals. *Vertical hairs* short, trifid. *Antenna* cylindrical, not markedly swollen, dark brown, with short stout spicules on the inner aspect rather more numerous towards the lower end. *Shaft hair* minute, simple, situated about  $\frac{1}{3}$  the distance from the base. *Apical hair* slightly longer than the paired blades, with about 2-5 very delicate branches.

*Thorax*. *Inner and middle shoulder hairs* with well-developed, fused chitinous bases. *Inner shoulder hair* rounded, with 20-30 branches. *Middle shoulder hair* more elongated, with 13-18 branches. *Outer shoulder hair* short, simple. *Thoracic palmate hair* well

developed, with 21–26 narrow leaflets which may be shouldered or serrated. Filaments about as long as blades. Between the thoracic palmate hairs lie two small, dark, oval sclerotized plates. *Pleural hairs* with medium-sized basal tubercles, spines short. Long *prothoracic* two simple and one feathered. *Mesothoracic* both simple or one with slight branching. *Metathoracic* one simple, one feathered. *Integument* without obvious spicules.

*Abdomen.* *Integument* without obvious spicules. *Palmate hairs* on segments I–VII all well developed. I with 15–16 shouldered leaflets, the filaments about  $\frac{2}{3}$  the length of the blades. II–VII with 15–20 broad leaflets, the filaments often as long as the blades.

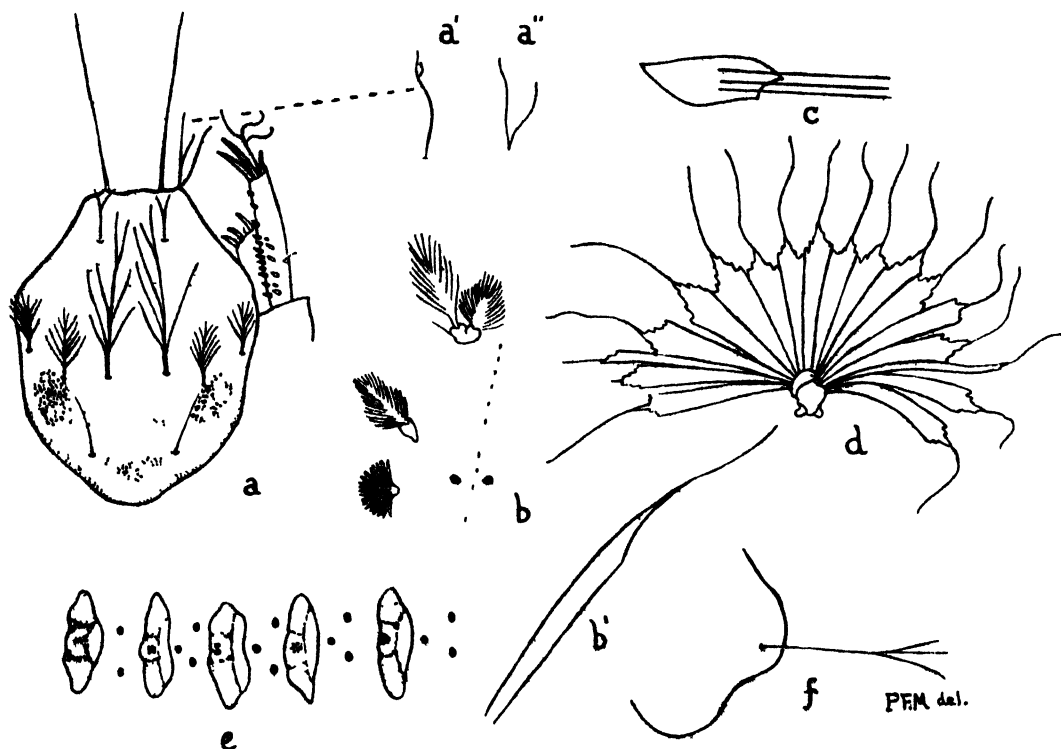


FIG. 1. *Anopheles domicolus* Edwards, larva. *a*.—Clypeus and antenna; *a'*, *a''*, variations in outer clypeal bristle in Kintampo and Kaduna specimens. *b*.—Thorax, showing position of shoulder hairs, thoracic palmate hair and metathoracic plates; *b'*, filament from thoracic palmate hair. *c*.—Base of metathoracic pleural hairs. *d*.—Typical abdominal palmate hair. *e*.—Tergal plates, segments I–V. *f*.—Saddle hair.

Average length of blade plus filament about 0.10–0.12 mm. *Lateral hairs* (hair 6) on segments IV–VI with 7–10 branches. Saddle hair split apically into 3–4 branches. The width of the *tergal plates* is about equal to the distance between the palmate hairs on segments VI and VII and about  $\frac{5}{6}$  of this distance on segments I–V. There are three *accessory tergal plates* on segments II–VII and two on segment I. The concave shape of the posterior border of the main tergal plates in the Kintampo specimen (Mattingly, 1944) is not apparent in the other specimens and appears to be an artefact due to crumpling of the pelt. *Pecten* dark and heavily sclerotized, with about 4–6 long teeth interspersed with 6–9 short ones, the latter mostly spiculate to half-way or beyond, the former with spicules present, if at all, only near the base. *Saddle hair* with 3–4 branches.

**BREEDING-PLACES.** Vegetation at stream edges. Dr. Walton informs me that he found the larvae also among long weeds in mid-stream in a remarkably rapid current.

**PUPA** (fig. 2). The nomenclature used in the following account is that employed by Evans (1938). *Paddle* with external border bare on about the basal half. Fringe spines slender, gradually passing into fine hairs towards the apex. No hairs visible beyond the terminal bristle. *Terminal bristle* hooked, between  $\frac{2}{5}$  and  $\frac{1}{2}$  the length of the paddle when fully extended. *Accessory paddle hair* short, with up to six very delicate branches. *Spine 'A'* on segment VIII with normal branching; on IV–VII very dark, sharp-pointed, not very strongly curved; on VII about  $\frac{1}{2}$  as long as segment VIII; on VI about the same length as on VII; on V about  $\frac{5}{6}$  as long as on VI; on IV about  $\frac{7}{10}$  as long as

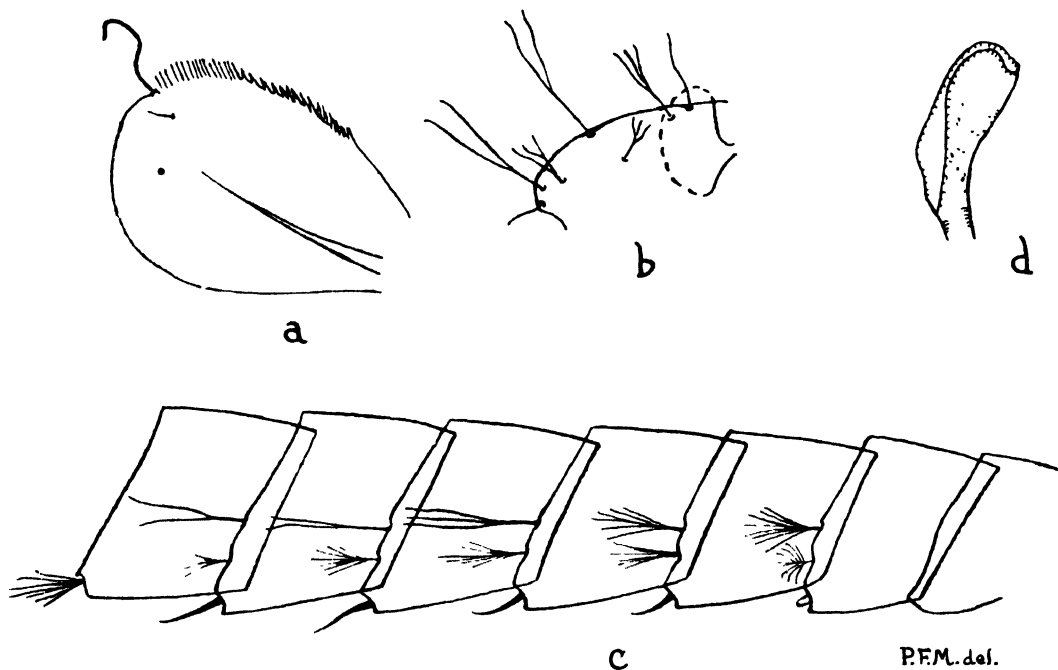


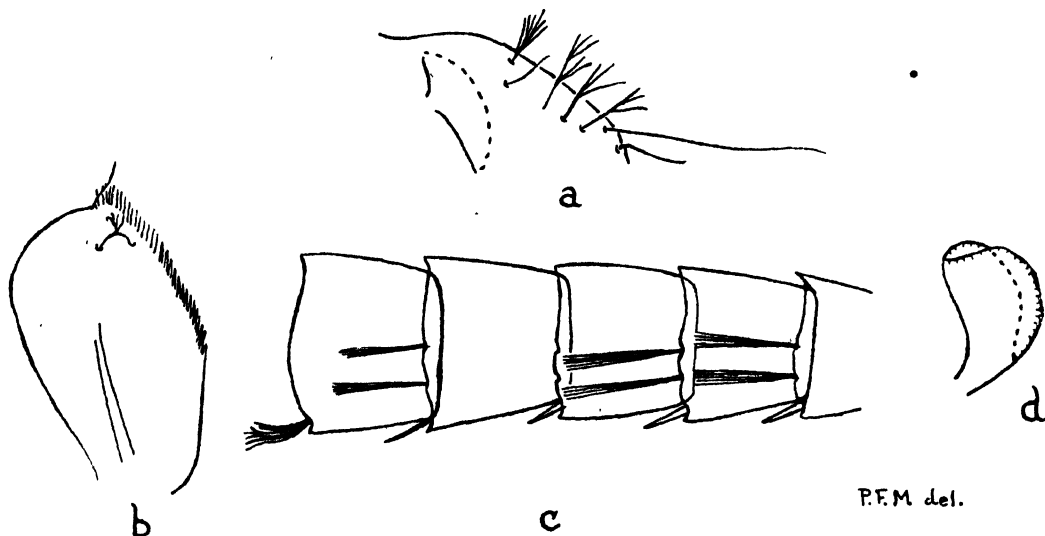
FIG. 2. *Anopheles domicolus* Edwards, pupa. a.—Paddle. b.—Segment I of abdomen. c.—Abdomen, segments II–VIII. d.—Respiratory trumpet.

on V; on III very small, blunt, pale in colour. *Seta 'B'* on VII with 6–7 branches, on VI with 6–8, on V with 6–9, on IV with 8–9, on III with 10–12. In all cases somewhat shorter and more slender than seta 'C.' *Seta 'C'* on V–VII dark and fairly stout, about  $\frac{3}{4}$  the length of the following segment; on IV rather more than  $\frac{1}{2}$  the length of the following segment; on III rather less; on VII single or bifid; on V and VI single to trifid; on IV with 5–7 branches; on III with 8–9 branches. *Segment I* with seta 'H' short and simple; 'K' with 2–5 branches, about equal in length to 'H'; 'L' very short, with 3–4 branches; 'M' bifid, of moderate length; 'S' 3–4 branched; 'T' 2–3 branched; 'U' short and simple. *Respiratory trumpet* slightly infuscated, especially in the region midway between base and apex. Meatus about  $\frac{1}{5}$  the length of the whole or rather less.

**RELATIONSHIPS.** Both the larva and pupa closely resemble the published descriptions of those of *A. michaeli* De Meillon and Leeson. The larva differs mainly in the clypeal markings, longer outer anterior clypeal hairs and better-developed abdominal palmate hairs, and in the possession of chitinized plates on the metathorax. The pupa differs mainly in having seta 'C' on segments VI and VII shorter than the following segments.

**Anopheles wilsoni** Evans (fig. 3)

The following account of the pupa is intended to supplement that given by Evans (1938), who mentions only setae 'B' and 'C' and the apical paddle hair. The description is based on a single pelt from Amani, Tanganyika Territory (Bagster Wilson). *Paddle* with narrow spines on the outer border down to about  $1/3$  the distance from the base, passing into fine hairs towards the apex. No hairs visible beyond the terminal bristle. *Terminal bristle* short and almost straight. *Accessory paddle hair* branched at half-way into four very delicate branches. *Spine 'A'* on segment VIII with normal branching ;



P.F.M. del.

FIG. 3. *Anopheles wilsoni* Evans, pupa. a.—Segment I of abdomen. b.—Paddle. c.—Abdomen, segments II–VIII. d.—Respiratory trumpet.

on IV–VII dark, sharp-pointed, unusually straight ; on III minute or absent. This spine on segment VII about  $1/3$  the length of the following segment, on VI about as long as on VII, on IV and V about  $5/6$  as long as on VI. *Seta 'B'* on VII about  $2/3$  the length of the following segment, on VI missing, on IV and V about as long as the following segments, on III rather shorter. All these with numerous, long, very fine branches. *Seta 'C'* about equal in length to 'B' on all segments on which the latter is present ; on VI about  $3/4$  the length of the following segment. On *Segment I* seta 'U' short and simple, seta 'T' very long and simple. *Respiratory trumpet* short and broad.

**Anopheles nili** Theobald (fig. 4, a)

Two pupal pelts from a collection of 15 made by Dr. J. Schwetz at Stanleyville, Belgian Congo, and presented by him to the British Museum, show a type of paddle fringe differing markedly from that normally encountered in this species. In these

specimens the whole of the paddle fringe on the outer side of the terminal bristle consists of stout spines, instead of the very slender, hairlike spines which are normally encountered. In the latter the base is relatively stout and spine-like, while the apex is drawn out into a long, very fine filament. When these filaments are broken off, the bases which remain look like short spines, although they are very much smaller than those noted in Dr. Schwetz's specimens. Dr. De Meillon informs me that he has a pelt resembling the latter and also from Stanleyville in the collection of the South African Institute for Medical Research at Johannesburg.

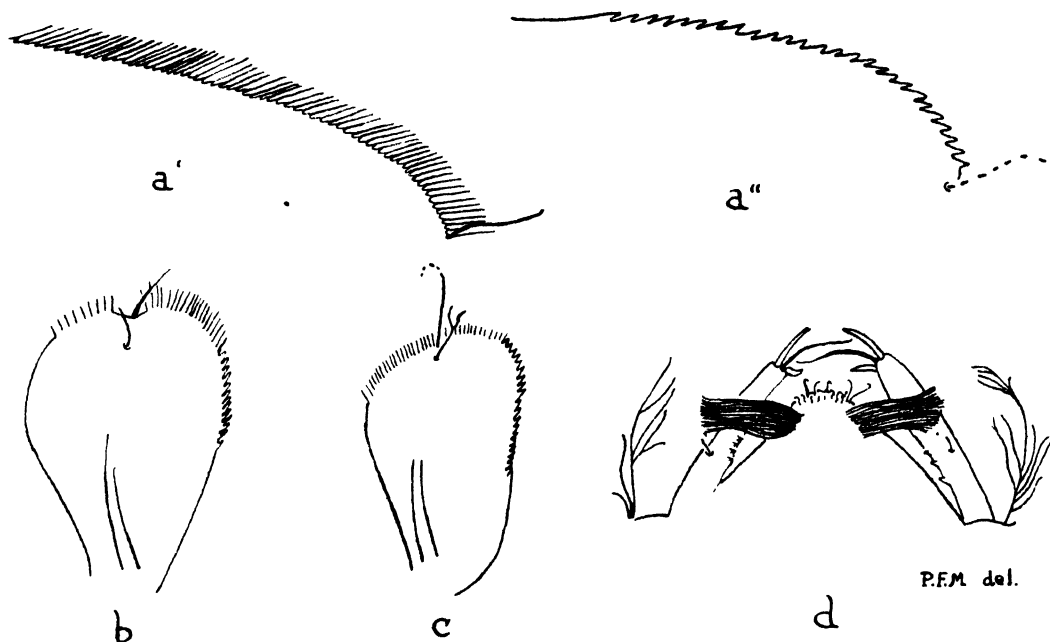


FIG. 4. *a*.—*Anopheles nili* Theobald, pupal paddle fringe; *a'*, normal, *a''*, aberrant form from Stanleyville. *b*.—*Anopheles hargreavesi* Evans, pupal paddle. *c*.—*Anopheles argenteolobatus* Gough, pupal paddle. *d*.—*Anopheles turkhudi* Liston, larval mouthbrushes.

### ***Anopheles hargreavesi* Evans and *Anopheles argenteolobatus* Gough**

In their original descriptions of the pupae of these species Evans (1938) and De Meillon (1929) suggest that the paddle fringe does not extend beyond the terminal bristle. Specimens now in the British Museum clearly show that it does so (fig. 4, *b-c*). It is not known whether this condition is constant, but it should be noted that in balsam mounts these very fine hairs are often difficult, if not impossible, to see. They can be seen clearly in mounts made with Puri's medium, of which the formula is given by Hopkins (1936).

### ***Anopheles multicolor* Cambouliu**

Senevet (1931) erred in his description of the third segment of the pupal abdomen. He has been followed by Christophers (1933) and Evans (1938). The true condition has been described and figured by Kirkpatrick (1925).



**Anopheles turkhudi** Liston (fig. 4, d)

Edwards, in a footnote to Evans (1938), suggests that *A. hispaniola* Theobald is a synonym of *A. turkhudi*. He bases this opinion on the non-existence of a supposed difference between the eggs, but ignores the very well-marked differences between the larvae and pupae of the two species. These differences, which appear to be both constant and significant, are as follows. PUPA with hairs clearly visible in the paddle fringe between the last spine and the terminal bristle in *A. hispaniola*; no hairs in this position in *A. turkhudi*. LARVA with the head normally very dark in *hispaniola*, although pale specimens are occasionally met with; in *turkhudi* the head is pale. In the latter species the *mouth-brushes* have a very peculiar and distinctive appearance, being shifted laterally so that they resemble those of some Culicini (see fig. 4, d, and an excellent photograph in Iyengar, 1930); in *hispaniola* they are of normal anopheline type. In *turkhudi* the *frontal hairs* have 2-6 branches; in *hispaniola* they have 6-13. In *turkhudi* the *palmate hairs* are very small, and are well developed only on abdominal segments IV-VI;\* in *hispaniola* they are larger, and are well developed on segments III-VII and present in a rudimentary condition on segment II. In the latter species the terminal filaments are more sharply pointed, and other minor differences have been noted. Edwards has been followed by Russell, Rozeboom and Stone (1943), who treat *hispaniola* as a synonym of *turkhudi*. In view of the existence of so many differences in the early stages this view does not appear to be tenable. Nor does it seem to be held by those who have recently encountered the two species in the field (Lumsden, 1944).

**Aedes (Mucidus) grahami** Theobald

Two conflicting accounts of the larva of this species have been published by Shield (1944) and Wolfs (1945). A pelt obtained by the present author at Takoradi, Gold Coast, is in some respects intermediate between the two, having about 40 comb-scales and the siphonal tuft almost exactly half-way along the siphon. It appears that the characters on which the larvae of this subgenus are usually separated are more variable than had previously been believed, and it is improbable that sufficient material is at present available for the construction of an absolutely reliable key. This is unfortunate, as *Aedes grahami*, although not very numerous in catches made in East Africa (Haddow *et al.*, 1947), has proved an important tree-top biter in Nigeria, and is accordingly of considerable interest to students of yellow fever. Wolfs (1945) suggests various criteria for distinguishing the larvae of *Aedes grahami* from those of other members of the group, but they do not appear to be reliable in all cases.

## LOCALITY RECORDS

The following records are the present author's, except where otherwise stated. The majority resulted from surveys carried out on behalf of No. 7 Malaria Field Laboratory, West African Force, between September, 1942, and December, 1944. New West African records of Anophelini resulting from such surveys and published by the author in a previous paper (Mattingly, 1944) are not repeated here. Separation into type-forms and varieties can only be assumed where the varietal name is given or the letters 't.f.' are appended to the specific name. Authorities for the names employed are in all cases those given by Edwards (1941).

\* Evans (1938), Christophers (1933) and Iyengar (1930) are all misleading on this point. The best account is given by Puri (1931).

## NORTHERN NIGERIA

## ILORIN

*Anopheles funestus* (M. D. Froud)

## KADUNA

*Anopheles brunnipes*  
 „ *coustani*  
 „ *domicolus*  
 „ *funestus*  
 „ *hancocki*  
 „ *maculipalpis*  
 „ *nili*  
 „ *pharoensis*  
 „ *pretoriensis*  
 „ *rufipes*  
 „ *squamosus*  
*Uranotaenia mashonaensis*  
*Taeniorhynchus africanus*  
 „ *uniformis*

*Aedes aegypti*  
 „ *simpsoni*  
*Culex annulioris*  
 „ *cinereus*  
 „ *decens*  
 „ *duttoni*  
 „ *guiarti*  
 „ *inconspicuosus*  
 „ *insignis*  
 „ *nebulosus*  
 „ *perfuscus*  
 „ *perfidiosus*  
 „ *tigripes*

## KANO

*Anopheles gambiae*  
 „ *obscurus*

*Anopheles rufipes*  
*Ficalbia splendens*

## LOKOJA

*Anopheles domicolus* (J. Y. Brown)*Megarhinus viridibasis* (P. J. Roche)

## MAIDUGURI (J. D. Robertson)

*Anopheles coustani* var. *ziemanni*  
 „ *funestus*  
 „ *gambiae*  
 „ *pharoensis*  
 „ *rufipes* t.f.  
 „ *squamosus*  
 „ *wellcomei*  
*Aedes aegypti*  
 „ *metallicus*

*Culex annulioris*  
 „ *decens*  
 „ *guiarti*  
 „ *poecilipes*  
 „ *tigripes*  
 „ *univittatus*  
*Ficalbia mimomyiaformis*  
 „ *plumosa*  
*Taeniorhynchus africanus*  
 „ *uniformis*  
*Uranotaenia balfouri*

## SOUTHERN NIGERIA

## ABA

*Anopheles gambiae*  
*Aedes aegypti*  
 „ *africanus*  
 „ *apicoargenteus*

*Culex cinereus*  
 „ *duttoni*  
 „ *nebulosus*

## AWBA

*Anopheles funestus* (M. D. Froud)

## ENUGU

*Anopheles coustani*  
 „ *funestus*  
 „ *gambiae*  
 „ *hancocki*  
 „ *moucheti* var. *nigeriensis*  
 „ *nili*  
*Megarhinus brevipalpis* spp. *conradti*  
*Hodgesia nigeriae*  
*Uranotaenia alboabdominalis*  
 „ *bilineata*

*Taeniorhynchus cristatus*  
 „ *uniformis*  
*Aedes aegypti*  
 „ *vittatus*  
*Culex annulioris*  
 „ *cinereus*  
 „ *decens*  
 „ *duttoni*  
 „ *nebulosus*  
 „ *perfidiosus*  
 „ *tigripes*  
 „ *tritaeniorhynchus*

## IBADAN (J. D. Robertson)

*Anopheles funestus*  
 „ *gambiae*  
 „ *nili*  
*Aedes aegypti*  
 „ *apicoargenteus*  
 „ *centropunctatus*  
 „ *luteocephalus*  
 „ *vittatus*  
*Culex annulioris*  
 „ *decens*  
 „ *duttoni*

*Culex guiarti*  
 „ *invidiosus*  
 „ *moucheti*  
 „ *nebulosus*  
 „ *poecilipes*  
 „ *pruina*  
 „ *rima*  
 „ *tigripes*  
*Eretmapodites chrysogaster* gp.  
*Ficalbia mimomyiaformis*  
*Taeniorhynchus africanus*  
*Uranotaenia annulata*

## IJBEBU ODE

*Anopheles cinctus*  
 „ *obscurus* var. *nowlini* (J. Y. Brown)

*Anopheles funestus* (M. D. Froud)

## OSHOGBO

*Anopheles funestus*  
 „ *gambiae*  
 „ *hargreavesi*  
 „ *nili*  
*Uranotaenia fusca*  
 „ *mashonaensis*  
*Ficalbia mimomyiaformis*  
 „ *uniformis*  
*Aedes fowleri*  
 „ *vittatus*

*Culex cinereus*  
 „ *cinerellus*  
 „ *duttoni*  
 „ *inconspicuus*  
 „ *ingrami* and var.  
 „ *perfidiosus*  
 „ *poecilipes*  
 „ *pruina* and var. *eschirasi*  
 „ *tigripes*

## PORT HARCOURT

*Anopheles gambiae*  
 „ *moucheti* var. *nigeriensis*  
 „ *obscurus*  
*Uranotaenia balfouri*  
 „ *chorleyi*  
 „ *ornata*  
 „ *pallidocephala*  
*Ficalbia mimomyiaformis*  
 „ *plumosa*  
*Aedes aegypti*  
 „ *simpsoni*

*Eretmapodites dracaenae*  
*Culex annulioris*  
 „ *cinerellus*  
 „ *decens*  
 „ *duttoni*  
 „ *macfie*  
 „ *nebulosus*  
 „ *perfidiosus*  
 „ *perfuscus*  
 „ *tigripes*

## LAGOS COLONY

## APAPA (J. D. Robertson)

*Anopheles coustani* var. *xiemanni*  
 „ *gambiae*  
 „ *melas*  
 „ *obscurus*  
 „ *pharoensis*  
*Uranotaenia annulata*  
 „ *balfouri*  
*Ficalbia plumosa*  
*Taeniorhynchus africanus*  
 „ *annetti*  
 „ *uniformis*  
*Aedes aegypti*  
 „ *africanus*  
 „ *albocephalus*  
 „ *argenteopunctatus*  
 „ *domesticus*

*Aedes irritans*  
 „ *nigricephalus*  
 „ *palpalis*  
*Culex cinerellus*  
 „ *decens*  
 „ *duttoni*  
 „ *fatigans*  
 „ *insignis*  
 „ *invidiosus*  
 „ *nebulosus*  
 „ *philipi*  
 „ *poecilipes*  
 „ *rima* gp.  
 „ *thalassius*  
 „ *tigripes*  
 „ *tritaeniorhynchus*

## IKEJA

*Anopheles cinctus* (J. Y. Brown)  
 „ *gambiae*  
 „ *jebudensis*  
 „ *moucheti* var. *nigeriensis*  
*Megarhinus brevipalpis* ssp. *conradti*  
*Hodgesia nigeriae*  
*Uranotaenia balfouri*  
 „ *chorleyi*  
 „ *pallidocephala*  
*Ficalbia uniformis*  
*Aedes aegypti*  
 „ *africanus*  
 „ *apicoargenteus*  
 „ *argenteoventralis* var. *dunni*  
 „ *kummi*  
 „ *longipalpis*

*Aedes luteocephalus*  
 „ *simpsoni*  
*Eretmapodites chrysogaster*  
*Culex albiventris*  
 „ *annulioris*  
 „ *cinereus*  
 „ *decens*  
 „ *duttoni*  
 „ *grahami*  
 „ *horridus*  
 „ *ingrami* var.  
 „ *invidiosus*  
 „ *perfidiosus*  
 „ *pruina* and var. *eschirasi*  
 „ *tigripes*

## YABA

*Anopheles coustani*  
 „ *gambiae*  
 „ *obscurus*  
*Hodgesia nigeriae*  
*Uranotaenia alboabdominalis*  
 „ *balfouri*  
 „ *caliginosa*  
 „ *coeruleocephala*  
 „ *fusca*  
 „ *nigromaculata*  
 „ *pallidocephala*  
 „ *philonuxia*  
*Aedomyia africana*  
*Ficalbia mimomyiaformis*  
 „ *nigra*  
 „ *uniformis*  
*Taeniorhynchus africanus*  
 „ *annetti*

*Taeniorhynchus aurites*  
 „ *metallicus*  
*Aedes aegypti*  
 „ *africanus*  
 „ *apicoargenteus*  
 „ *circumluteolus*  
 „ *domesticus*  
 „ *longipalpis*  
 „ *luteocephalus*  
 „ *simpsoni*  
*Eretmapodites chrysogaster* gp.  
*Culex annulioris*  
 „ *cinereus* var. *uniformis*  
 „ *decens*  
 „ *fatigans*  
 „ *guiarti*  
 „ *nebulosus*  
 „ *thalassius*  
 „ *tigripes*

## GOLD COAST, NORTHERN TERRITORIES

## DABOYA

*Anopheles gambiae*  
 „ *wellcomei* (L. Berners)

*Culex annulioris*  
 „ *decens*  
 „ *univittatus*

## KINTAMPO

*Anopheles coustani*  
 „ *domicolus*  
 „ *freetownensis*  
 „ *funestus*  
 „ *gambiae*  
 „ *maculipalpis*  
 „ *rufipes* var. *ingrami*

*Uranotaenia annulata* var. *apicotaeniata*  
 „ *balfouri*  
 „ *mashonaensis*  
*Aedes tarsalis*  
*Culex annulioris*  
 „ *decens*  
 „ *duttoni*  
 „ *ingrami*  
 „ *tigripes*

## NASIA

*Anopheles wellcomei* (L. Berners)

## NUNGWA

*Anopheles squamosus* (L. Berners)

## PONG TAMALE

*Anopheles gambiae*

## TAMALE

*Anopheles funestus*,  
" *gambiae*

*Culex annulioris*  
" *univittatus*

## YEJI

*Anopheles wellcomei* (L. Berners)

## ASHANTI

## KUMASI

*Anopheles cinctus*  
" *coustani* and var. *ziemanni*  
" *funestus*  
" *gambiae*  
" *obscurus*  
*Megarhinus brevipalpis* ssp. *conradti*  
*Hodgesia nigeriae*  
*Ficalbia hispida* var. *sunyaniensis*  
*Aedes abnormalis*  
" *aegypti*  
" *centropunctatus*  
" *cummini*  
" *grahami*  
" *palpalis*

*Aedes punctothoracis*  
" *simpsoni*  
" *tarsalis*  
*Eretmapodites chrysogaster* gp.  
" *grahami*  
*Culex albiventris*  
" *annulioris* and var. *consimilis*  
" *grahami*  
" *ingrami*  
" *invidiosus*  
" *nebulosus*  
" *perfuscus*  
" *tigripes*

## GOLD COAST COLONY

## ACCRA

*Aedes circumluteolus*  
" *cummini*  
" ? *dalzieli*  
" *hirsutus*  
" *scatophagoides*  
*Culex ethiopicus*  
" *fatigans*  
" *perfidiosus*  
" *univittatus*  
(J. D. Robertson)  
*Anopheles coustani*  
" *funestus*  
" *gambiae*

*Anopheles hargreavesi*  
" *pharoensis*  
*Uranotaenia balfouri*  
*Aedomyia africana*  
*Ficalbia mimomyiaformis*  
*Taeniorhynchus africanus*  
*Aedes aegypti*  
" *albocephalus*  
" *fowleri*  
*Culex inconspicuus*  
" *invidiosus*  
" *poecilipes*  
" *thalassius*

## ASUBOI

*Anopheles funestus*  
" *gambiae*  
" *hargreavesi*  
*Uranotaenia annulata* var. *apicotaeniata*  
" *ornata*  
*Ficalbia mimomyiaformis*  
" *pallida*  
" *uniformis*  
*Taeniorhynchus africanus*  
*Aedes aegypti*  
" *apicoargenteus*

*Aedes metallicus*  
" *simpsoni*  
*Eretmapodites chrysogaster*  
" *dracaenae*  
" *quinquevittatus*  
*Culex albiventris*  
" *guiarti*  
" *ingrami*  
" *invidiosus*  
" *nebulosus*  
" *tigripes*

## AXIM (M. D. Froud)

*Anopheles cinctus*  
" *funestus*  
" *gambiae*  
" *hargreavesi*  
" *obscurus* and var. *novlini*

*Uranotaenia balfouri*  
" *bilineata*  
" *chorleyi*  
" *mashonaensis*  
*Hodgesia nigeriae*

## AXIM (con.)

*Ficalbia mimomyiaformis*  
 " *splendens*  
*Eretmapodites chrysogaster*  
 " *grahami*  
*Culex guarti*  
 " *grahami*  
 " *inconspicuus*

*Culex ingrami*  
 " *nebulosus*  
 " *pruina* var. *eschirasi*  
 " *rima*  
 " *thalassius*

## TAKORADI

*Anopheles coustani* var. *ziemanni*  
 " *obscurus*  
 " *paludis*  
*Hodgesia psectropus*  
*Aedes domesticus*  
 " *palpalis* ssp. *carteri*  
 (J. D. Robertson)  
*Anopheles funestus*  
 " *gambiae*  
 " *pharoensis*  
*Uranotaenia annulata* var. *apicotaeniata*  
 " *balfouri*  
 " *nigromaculata*  
*Taeniorhynchus africanus*  
*Aedes aegypti*  
 " *albocephalus*  
 " *cumminsi*  
 " *fowleri*  
 " *fraseri*

*Aedes furcifer* (or *taylori*)  
 " *grahami*  
 " *irritans*  
 " *nigricephalus*  
 " *punctothoracis*  
 " *scatophagoides*  
 " *stokesi*  
*Eretmapodites dracaenae*  
*Culex annulioris* and var. *consimilis*  
 " *duttoni*  
 " *fatigans*  
 " *guarti*  
 " *horridus*  
 " *inconspicuus*  
 " *ingrami*  
 " *invidiosus*  
 " *nebulosus*  
 " *philipi*  
 " *thalassius*

## SIERRA LEONE

## Bo

*Aedes argenteopunctatus* (J. D. Robertson)

## FREETOWN (G. A. Walton)

*Anopheles domicolus* *Anopheles marshalli*  
 (See also under *Uranotaenia mashonaensis* below)

## NOTES

*Anopheles cinctus*. Mr. Froud's record from Axim is the first from Gold Coast Colony.

*Anopheles coustani*. The record of the type-form from Kumasi is the first from Ashanti.

*Anopheles domicolus*. Dr. Walton's record from Freetown appears to be the first from Sierra Leone.

*Anopheles jebudensis*. This species was taken for the first time at Ijebu Ode, Southern Nigeria, by Froud (1944). It has since been found again by the author breeding in a heavily shaded stream-edge at the bottom of a ravine near Ikeja.

*Anopheles marshalli*. Dr. Walton informs me that he bred out type-form *A. marshalli* Theo. from larvae taken together with those of *A. hancocki* among floating vegetation in clean water with some current in the reservoir at Waterloo aerodrome near Freetown. Mr. Brown's record from Nigeria (Mattingly, 1944) and a doubtful one from the same colony given by Evans (1938) are the only previous notifications of this species from West Africa.

*Anopheles melas*. It is understood from Dr. Robertson that his diagnostic character for this species is intended to apply only to the fore tarsus when viewed from the dorsal surface, and not to the hind tarsus, as stated by the author (Mattingly, 1944). The error is regretted.

*Anopheles wellcomei*. The records of this species from Daboya, Nasia and Yeji are due to Major Lewis Berners, of the United States Army. They are believed to be the first from the Gold Coast.

*Hodgesia nigeriae*. Edwards (1941) gives records of this species from Nigeria only, apart from a doubtful one from Sierra Leone (Freetown, Fraser). Davey (1939) gives another record from the latter colony which Edwards apparently overlooked. Adults were bred out from larvae taken by the present author at Kumasi, Gold Coast, and Dr. Robertson states in a personal communication that he has had larvae from Kumasi which appeared to be *nigeriae*. Froud's record from Axim is based on larvae, one of which is now in the British Museum.

*Hodgesia psectropus*. Dr. Robertson's record of this species from the Gold Coast is believed to be the first from outside the Belgian Congo.

*Uranotaenia alboabdominalis*. Larvae of this species were taken at Enugu, Nigeria, and a single adult in a house at Yaba. The only previous West African record is from the Gold Coast.

*Uranotaenia balfouri*. Dr. Robertson's record from Maiduguri appears to be the first from Northern Nigeria.

*Uranotaenia bilineata*. The record from Enugu is the first from Nigeria.

*Uranotaenia chorleyi*. Larvae of this species taken by Froud at Axim and Ijebu Ode (Froud, 1944) and by the author at Ikeja and Port Harcourt are the first recorded from West Africa. All the records are based on larvae only and require confirmation, as there are several West African *Uranotaenia* whose larvae have yet to be described.

*Uranotaenia mashonaensis*. Edwards (1941) does not list this among the West African species, although Ingram and Macfie (1924) recorded it from Accra and Davey (1939) from Sierra Leone. Other Sierra Leone records by Davey omitted by Edwards from his table are *Ficalbia splendens*, *Aedes filicis* and *haworthi*, and *Culex guiarti*, *moucheti* and *weschei*. Large numbers of adult *U. mashonaensis* were bred out by the author at Oshogbo, Nigeria, and the records from Kaduna in Northern Nigeria, Axim in Gold Coast Colony and Kintampo on the northern border of Ashanti appear to indicate that it is widespread in British West Africa.

*Uranotaenia ornata*. The record from Asuboi appears to be the first from Gold Coast Colony.

*Uranotaenia pallidocephala*. Of this species Edwards (1941) says that 'records require confirmation.' Larvae were taken by the author at Yaba, Ikeja and Port Harcourt.

*Ficalbia splendens*. Larvae taken from a weedy borrow-pit at Kano are believed to be the first from Northern Nigeria.

*Taeniorhynchus cristatus*. Adults of this species were taken in European quarters at Enugu during November, 1942. The only previous West African record is from Sierra Leone.

*Aedes cumminsi* and *hirsutus*. Dr. Robertson's records from Accra are the first from Gold Coast Colony.

*Aedes dalzielii*. Dr. Robertson's record from Accra, the first from the Gold Coast, is a doubtful one and requires confirmation.

*Aedes fowleri*. The records from Takoradi and Accra are the first from Gold Coast Colony, although the species has been recorded from the Northern Territories.

*Aedes furcifer*. In Southern Nigeria, as in the Sudan (Lewis, 1945) and Northern Rhodesia (De Meillon, 1943), adult females of this species have proved indistinguishable from those of *A. taylori*. The record from Takoradi therefore requires confirmation.

*Aedes mucidus*. Dr. Robertson's record from Takoradi is based on larvae only. In view of the similarity now known to exist between the larva of this species and that of *A. grahami*, and the fact that *A. mucidus* has not previously been recorded from West Africa, this record requires confirmation.

*Aedes punctothoracis*. The record from Kumasi is the first from Ashanti.

*Eretmapodites dracaenae*. The records from Asuboi and Takoradi are the first from Gold Coast Colony.

*Culex albiventris*. The record of this species from Ikeja is believed to be the first from Nigeria.

*Culex annulioris*. The records from Kaduna and Maiduguri are the first from Northern Nigeria. The record of the type-form from Kumasi is the first from Ashanti.

*Culex ethiopicus*. Dr. Robertson's record from Accra is the first from Gold Coast Colony.

*Culex ingrami*. Larvae both of the Gold Coast type and of the Kampala variety (Hopkins, 1936) were taken and bred out at Oshogbo. Larvae of the Gold Coast type were taken at Asuboi and Takoradi. It appears, therefore, that the Gold Coast type occurs in Gold Coast Colony as well as in Ashanti, and that both type-form and variety occur in Nigeria. Previous records are from Ashanti only.

*Culex macfie*. The record from Port Harcourt is the first from Southern Nigeria.

*Culex pruina* var. *eschirasi*. Numerous larvae were taken together with those of the type-form at Oshogbo and Ikeja, Southern Nigeria. Previous records are from Gaboon only.

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# THE PERIOD REQUIRED BY *LITOMOSOIDES CARINII* TO REACH THE INFECTIVE STAGE IN *LIPONYSSUS BACOTI*, AND THE DURATION OF THE MITES' INFECTIVITY

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## INTRODUCTION

It was originally shown by Williams and Brown (1945, 1946) that the mite *Liponyssus bacoti* is a vector of the filaria worm *Litomosoides carinii* to cotton rats and white rats. This observation by the American workers has been confirmed and amplified by Scott and Cross (1946), Hawking and Burroughs (1946), and Bertram, Unsworth and Gordon (1946). These authors and Williams (1946) and Scott, Stembridge and Sisley (1947) obtained transmission by accommodating together, or by interchanging, infected cotton rats and uninfected animals in mite-infested artificial rat-nests, or by transferring numbers of mites from the nests of infected rats to uninfected hosts, the mites generally being infected by allowing them to feed for periods of at least 10 days on the infected animals. The occurrence in the mite of developmental stages of the worm was first reported by Williams and Brown (1945), who later (1946) considered the infective form to be between  $800\mu$  and  $1,000\mu$  in length, though no information was recorded indicating the time required for these infective forms to develop. Hawking (1947), in a footnote to a review of Bertram *et al.* (1946), comments that in his experiments transmission always occurred when a period of 21 days was allowed to elapse between the mites' first meal on an infected animal and the last meal on an uninfected animal. More recently, Scott *et al.* (1947) have reported that in routine transmission work they allow mites to feed and breed for 1-2 weeks on an infected cotton rat and then remove the rat from its artificial nest. Those mites which are still alive two weeks afterwards and which contain active worm larvae are assumed to have become infective. Of samples of 209 and 180 mites from such nests, 20 and 24 per cent, respectively were seen to contain active worm larvae when the live mites were examined in a drop of saline under a cover-slip with a compound microscope. From these observations, in which the date of a mite's first infecting meal is not accurately known, the interval required for mites to become infective appears to be within the limits of two and three weeks after the mites first become infected with microfilariae.

Bertram *et al.* (1946) give an account of methods by which the date on which mites first become infected is known within a limit of 24 hours. In one experiment a series of female mites was infected from a cotton rat during a single 24-hour period and was subsequently maintained for 33 days on the scarified tail of a white rat. The same rat was used for all the meals provided for these mites, the mites being induced to feed approximately every five days. Dissections of the mites were few, and active worm-like

forms, varying from  $420\mu$  to  $935\mu$  in length, were obtained only from the 20th to the 33rd day after the mites' infecting meal, mites dissected before the 20th day proving negative for developmental forms of the worm, except for a worm stage on the seventh day. At the time when this paper was published the authors did not know if transmission had been achieved in this experiment, but they thought that some of the active worms were probably infective, since Williams and Brown (1946) considered that infective forms ranged between  $800\mu$  and  $1,000\mu$  in length. Later, the rat was found to be positive for 1-8 microfilariae in five out of 24 peripheral blood films, and on dissection the rat was found to contain one female and one male worm of *L. carinii* in the right pleural cavity. This result showed that the infected mites had transmitted the infection to the rat and that transmission occurred in association with the feeding-habits of the mite; but the time required for the mites to become infective and the duration of their infectivity were still undetermined. After the evidence of transmission had been obtained, experiments were undertaken to investigate these points. Female mites infected by feeding during one 24-hour period on an infected cotton rat were subsequently fed in small batches on different white rats and cotton rats at approximately five-day intervals over a period lasting up to 35 days. It was hoped to obtain the required information from the subsequent dissection of the white rats and by the dissection or blood examination of the cotton rats at an appropriate interval after the single occasion of the host's exposure to possible infection by the mites. At the same time it was intended that dissections should be made of mites of the same series in order to relate the transmission results in the rodents with the developmental stages of the worm occurring in the mites at the time of particular blood-meals.

The results of the experiments are recorded below.

## METHODS

Only adult female mites were used; they were bred out for each experiment from deutonymphs which had engorged as protonymphs on uninfected cotton rats. Their infecting meal was their first meal as adults and was obtained by leaving them free to feed for 24 hours on an infected cotton rat in a cage set in a sawdust-tray mounted over water. When subsequent meals were taken on uninfected cotton rats the same apparatus was used for a similar period, while in the case of white rats the tail was scarified and the mites were free to feed on it for about one hour. Between meals the mites were kept at a temperature of  $23-25^{\circ}\text{C}$ . in glass tubes plugged with cotton wool. These methods are described in detail by Bertram *et al.* (1946).

It will be observed that two figures are given in Tables I, II, III and V for the number of mites in contact with a rat on the occasion of a meal. The higher figure is the number of mites released on a cotton rat for 24 hours or, in the case of white rats, free to run in contact with the scarified tail for a period of usually about one hour. The lower figure represents the number of mites which engorged on a white rat's tail or were recovered gorged from a cotton rat host. While there is evidence that transmission occurs in association with the feeding-habits of the mite, information is lacking as to the actual mode of transmission. Consequently it must be conceded that mites not recovered from a cotton rat may have been responsible for transmission, and that, in the case of the white rats, transmission might have followed the close contact of unfed mites with the raw tissue of the scarified tail. In the absence of knowledge on such points it seems

advisable to record the maximum number of mites free to come into contact with the host as well as the number known to engorge upon it.

Developmental stages of the worm larva were found by teasing mites apart with fine needles in a drop of dissection-medium on a slide under a low-power binocular microscope, and by examining the slide under the usual powers of a compound microscope. Tap-water, serum, normal saline, and 5 per cent. glucose solution were used as media. Camera-lucida outlines were made of living and of dried preparations of the active form of the larva found from the 14th day onwards, and their lengths were measured at leisure.

Reference has already been made to the small number of microfilariae found in the peripheral blood of an infected white rat, and, since Williams and Brown (1946) have shown that developing worms occur in the pleural cavity of white rats as early as 42 days after exposure, evidence of transmission to the rodents was obtained from the dissection of the white rats at intervals of 47–81 days after their exposure to possible infection. Cotton rats were also dissected if they failed to become positive for microfilariae in the peripheral blood-stream by the 60th day after exposure.

### THE SEQUENCE OF THE EXPERIMENTS

Three series of mites were infected and maintained on cotton rats and white rats by the methods described.

The experiments could have been conducted with cotton rats only, but it was decided to utilize white rats for the earlier meals in the first experiment. The use of cotton rats involves the liberation of mites on the rat for 24 hours, and the number of mites recovered is always less—sometimes considerably so—than the number released. When, however, mites are fed on the scarified tail of a white rat there is a limited loss, owing to damage in manipulation or to the failure of some mites to feed satisfactorily. Economy in the loss of mites by the use of white rats enabled a series of mites to be maintained in adequate numbers for a longer period than might have been possible with cotton rats as hosts for every meal. In the first experiment (Table I) varying numbers of white rats were therefore used for the mites' first meal on the fourth day to their seventh meal on the 35th day after their infecting meal. On the occasions, however, of the 15th, 20th and 25th days after the mites' infecting meal a cotton rat was also exposed to infection, this period being considered as most likely to coincide with the infective phase of the mites. A second experiment (Table II) was undertaken within 13 days of the commencement of the first, and the infected cotton rat of the first experiment was again used as a source of infection to the mites. This series of mites was fed on white rats on the fourth and ninth days only after their infecting meal, and the mites were then dissected on the 14th–16th day to obtain a percentage infection-rate without the third—and presumed first infective—meal being offered to them. In the third experiment (Table III) cotton rats only were used as hosts, to confirm the evidence which had by this time been obtained from white rat hosts in the earlier meals of the first and second experiments.

### RESULTS

#### (a) *The Transmission of Litomosoides carinii to the Rats*

The results obtained from the rodents in the three experiments are shown separately in Tables I, II and III and are summarized in Table V. It will be seen from the tables

that successful transmission did not occur to six white rats and one cotton rat nor to seven white rats and two cotton rats exposed to possible infection on the 4th-5th days and on the 9th-10th days respectively after the mites' infecting meal. White rats and cotton rats, however, both became infected when exposed to possible infection on the occasion of the mites' third meal, or on the 15th day, after their infecting meal.

TABLE I

Showing the results obtained in white rats and cotton rats exposed to infection with *L. carinii* by small batches of infected female *L. bacoti*. The mites were released to feed on the rats at approximately five-day intervals until their seventh meal on the 35th day after engorging during 24 hours on 19-20.11.47 on cotton rat no. 25, which showed 2,584 mf./c.mm. of peripheral blood. Of 29 of the mites dissected prior to their first transmission 38 per cent. were positive for 1-5 worms:

Date	Mites' blood-meals		No. of mites		Host	Time of dissection (D) or first positive blood films (B.F.) in days after exposure to possible infection	Transmission result
	No. after infecting meal	Time in days after infecting meal	(a) Released on host	(b) Engorged on host			
23.11.46	1st	4th	20	20	White rat 1	72 days (D)	Negative
			20	13	" " 2	72 days (D)	Negative
			20	20	" " 3	—	— <sup>1</sup>
			20	19	" " 4	72 days (D)	Negative
			20	13	" " 5	72 days (D)	Negative
			25	24	" " 6	—	— <sup>2</sup>
28.11.46	2nd	9th	20	14	White rat 7	81 days (D)	Negative
			12	12	" " 8	"	Negative
			20	20	" " 9	"	Negative
			20	19	" " 10	"	Negative
			20	13	" " 11	"	Negative
			24	16	" " 12	"	Negative
4.12.46	3rd	15th	25	23	White rat 13	77th day (D)	Positive <sup>3</sup>
			24	18	" " 14	76th day (D)	Positive <sup>4</sup>
			30	17	Cotton rat 80	B.F. + on 54th day	Positive <sup>5</sup>
9.12.46	4th	20th	25	20	White rat 18*	81st day (D)	Negative
			30	19	Cotton " 77	B.F. + on 53rd day	Positive <sup>6</sup>
14.12.46	5th	25th	13	10	White rat 21	76th day (D)	Negative
			13	3	Cotton " 78	44th day (D)	Positive <sup>7</sup>
19.12.46	6th	30th	13	9	White rat 22	71st day (D)	Negative
24.12.46	7th	35th	8	7	White rat 23	60th day (D)	Negative

\* See Table II for white rats 16, 17 and 19. White rats 15 and 20 are not relevant to these experiments.

<sup>1</sup> Died 4 days after exposure.

<sup>2</sup> Died 18 days after exposure.

<sup>3</sup> Three ♀, two ♂ worms in pleural cavities.

<sup>4</sup> Four ♀, one ♂ worm in pleural cavities.

<sup>5</sup> Average of 275 mf./c.mm. of peripheral blood (range 90-670 mf./c.mm.) in 20 weekly counts.

<sup>6</sup> Average of 97 mf./c.mm. of peripheral blood (range 40-190 mf./c.mm.) in 18 weekly counts.

<sup>7</sup> Rat died. One ♂ worm in pleural cavity.

There is some suggestion from the results obtained in the cotton rats that, although transmission occurs first on the 15th day after the mites' infecting meal, it continues, with diminishing intensity, on the 20th day and on the 25th day. Thus, 20 counts from

TABLE II

Showing the results obtained in white rats exposed to infection with *L. carinii* by small batches of infected female *L. bacoti*. The mites were released to feed on the rats on the fourth and ninth days after their infecting meal during 24 hours on 2-3.12.46 on cotton rat no. 25, which showed 3,000 mf./c.mm. of peripheral blood. Of 37 mites dissected on the 14th-16th day after the infecting meal 21.8 per cent. were positive for 1-2 worms

Date	Mites' blood-meals		No. of mites		Host	Time of dissection in days after exposure to possible infection	Transmission result
	No. after infecting meal	Time in days after infecting meal	(a) Released on host	(b) Engorged on host			
6.12.46	1st	4th	13 29	12 27	White rat 16 " " 17	60th day 60th "	Negative Negative
11.12.46	2nd	9th	39	39	White rat 19	47th day	Negative

cotton rat 80 taken at approximately weekly intervals during the first five months after the rat had become positive range from 90 to 670 mf./c.mm. of peripheral blood, with an average for the 20 counts of 275 mf./c.mm. In the case of cotton rat 77, which was exposed to infection by a similar number of the same series of mites five days later, the average for 18 counts over a similar period is 97 mf./c.mm., with a range of from 40 to 190 mf./c.mm. of peripheral blood. In the case of cotton rat 78, which was exposed to infection on the 25th day after the mites' infecting meal, a single male worm was found on dissecting the rat following its death on the 44th day after exposure to infection. This low infection may, however, be due to the smaller number of mites available for release on the rat. Successful transmission to white rats was not apparent, except on the occasion of the meal taken by the mites 15 days after their infecting meal.

(b) *The Appearance of Microfilariae in the Peripheral Blood of Cotton Rats*

The blood of cotton rat 80, in the first experiment (Table I), was found negative for microfilariae on the 30th, 48th and 51st days after the rat's single day's exposure to

TABLE III

Showing the results obtained in cotton rats exposed to infection with *L. carinii* by small batches of infected female *L. bacoti*. The mites were released to feed on the cotton rats on the 5th, 10th, 15th and 20th days after the mites' infecting meal during 24 hours on 18-19.3.47 on cotton rat no. 26, which showed 600 mf./c.mm. of peripheral blood. A percentage infection-rate was not determined for this series of mites

Date	Mites' blood-meals		No. of mites		Host	Time of dissection (D) or first positive blood film (B.F.) in days after exposure to possible infection	Transmission result
	No. after infecting meal	Time in days after infecting meal	(a) Released on host	(b) Engorged on host			
23.3.47	1st	5th	120	95	Cotton rat 95	102 days (D)	Negative
28.3.47	2nd	10th	55 40	18 11	Cotton rat 111 " " 112	97 days (D) 97 days (D)	Negative Negative
2.4.47 7.4.47	3rd 4th	15th 20th	29 10	10 2	Cotton rat 119	B.F. + on 51st day	Positive

infection, but became positive by the 54th day. Cotton rat 77, which was also exposed to infection on a single occasion (Table I), became positive for microfilariae in the peripheral blood on the 53rd day, being negative in previous films on the 31st, 43rd, 46th and 49th days after exposure to the mites. Cotton rat 119 was exposed to infection twice (Table III), and the peripheral blood was first found to be positive for microfilariae on the 51st day after the first exposure. These results for the lapse of time before infected cotton rats have demonstrable microfilariae in the peripheral blood agree closely with the minimum estimate of 50 days given by Scott (1946) in cotton rats exposed to infection by a colony of infected mites for a period of 10 days.

The results for all the rats used in the three experiments are summarized in Table V, figures for the intensity of infection in cotton rats being derived from the first experiment only, and the figures for the mites being derived from the data for all three experiments.

TABLE IV

Showing the results obtained in the dissections of 41 female *L. bacoti* at intervals after their infecting meal on a cotton rat. This series of mites is a sample of the mites infected during 24 hours on 19-20.11.47 on cotton rat no. 25, and subsequently maintained by seven meals over a period of 36 days on the white rats and cotton rats shown in Table I

Date	No. of mites dissected	Time of dissection in days after mites' infecting meal	No. of mites positive	No. of worm larvae per mite	Notes on worm larvae
26.11.46	1	7th	Nil	—	—
28.11.46	6	9th	3	2, 4, 5	154-180 $\mu$ long, inactive ; with sickle-shaped tail Active thread-like larvae ; lengths between about 0.5 and 1 mm., but variable in individual worms Ditto Ditto
29.11.46	8	10th	1	1	
3.12.46	9	14th	4	1, 1, 2, 2	
4.12.46	1	15th	1	5	
5.12.46	4	16th	2	2, 4	Ditto
Above this line mites had not been offered their third and infective meal					
9.12.46	4	20th	2	1, 2	Ditto
10.12.46	6	21st	Nil	—	—
to		to			
24.12.46	2	35th	1	1	Similar to active larvae of 14th day
25.12.46		36th			

(c) *Developmental Forms of L. carinii in Dissections of Mites Maintained on the Rats of Experiment 1 and Experiment 2*

Table IV shows the results obtained from the dissections of 41 mites at different times after they became infected from cotton rat 25 containing 2,584 mf./c.mm. of peripheral blood, and maintained subsequently for 36 days on the white rats and cotton rats of experiment 1 (Table I). Of 29 mites dissected before transmission had occurred, as observed in the rat hosts, 38 per cent. were positive for 1-5 developmental forms of the worm. Of 12 mites dissected after transmission could have occurred at least once, three were positive, and one of these mites contained one active worm larva as late as the 36th day after the mite's infecting meal. The mites of the second experiment (Table II) were infected on the same cotton rat 14 days later than those of the first experiment. The blood count was at this time higher—3,000 mf./c.mm. of peripheral blood—and of the

37 mites of the second series dissected on the 14th–16th day of the worm's development eight were positive (21·8 per cent.) for 1–2 active larval forms of the worm. No mite dissections were made in the third experiment, owing to the unusually heavy loss of mites during their periods of freedom to feed on the cotton rats.

TABLE V

Summarizing the data of Tables I–IV, showing that cotton rats and white rats, exposed to infection with *L. carinii* by infected female *L. bacoti*, are first infected at the third meal or on the 15th day after the mites' infecting meal, and that transmission continues, but appears to diminish, on the 20th and 25th days (fourth and fifth meals). Infective forms of the worm occur apparently as early as the 14th day of their larval development, and appear to persist in the mite until as late as the 36th day after its infecting meal

Mites' blood-meals		Type of host	No. of hosts used	No. of mites		Transmission results	Developmental stage of worm in mites
No. after infecting meal	Time in days after infecting meal			(a) Released on hosts	(b) Engorged on hosts		
1st	4th–5th	White rats Cotton „	6 1	29 120	12 95	Negative Negative	
2nd	9th–10th	White rats Cotton „	7 2	39 55	12 11	Negative Negative	Length 150–180 $\mu$ ; inactive, with sickle-shaped tails
3rd	15th	White rats Cotton „	2 2	25 30	18 10	Positive (5 worms per rat) Positive ; average of 20 weekly counts = 275 mf. / c.mm. of peripheral blood	14th – 36th day Active thread-like larvae varying in length from about 0·5 to 1 mm. Some or all infective forms
4th	20th	White rats Cotton „	1 1	25 30	20 19	Negative Positive ; average of 18 weekly counts = 97 mf. / c.mm. of peripheral blood	
5th	25th	White rats Cotton „	1 1	13 13	10 3	Negative Positive (1 ♂ worm)	
6th	30th	White rats	1	13	9	Negative	
7th	35th	White rats	1	8	7	Negative	

The worm larvae observed on the ninth and tenth days of their development were inactive and ranged from 154  $\mu$  to 180  $\mu$  in length when alive in normal saline. They possessed a short sickled-shaped tail, as described by Williams and Brown (1945) in dissections of mites collected at random from infected rats. The larvae obtained in the dissection of mites on the 14th–16th day after their infecting meal were long, active, thread-like worms capable of twisting and coiling vigorously. Camera-lucida measurements of the lengths of a number of these larvae alive gave extremely variable results, ranging from 550  $\mu$  to 690  $\mu$  in 5 per cent. glucose solution, 680  $\mu$  in serum, 660–940  $\mu$  in normal saline, and 1,004  $\mu$  when dissected in tap-water. In one dissection in normal saline of a larva



in its 14th day of development, the length, while it was alive, was recorded by camera-lucida outline as between  $450\mu$  and  $500\mu$  when the worm was near the edge of the saline drop. On being moved, without apparent injury, to the centre of the drop this larva lengthened to about  $800\mu$ . Considerable contraction occurs on allowing preparations of 14-16-day-old larvae to dry, measurements of length then varying between at least  $410\mu$  and  $950\mu$ . Worm larvae seen in dissections from the 20th to the 36th day after the mites' infecting meal appeared to be similar in form and activity to those found from the 14th to the 16th days.

### SUMMARY AND CONCLUSIONS

It is concluded that white rats and cotton rats which become positive for *L. carinii* after serving as hosts to infected female *L. bacoti* are infected by the transmission of the active thread-like worm larvae which develop in the mites. Transmission appears to occur in association with the feeding-habits of the mite, but the actual mode of transmission is not known, and other methods of transmission by the mite cannot be discounted.

Infected mites stored at 23-25° C. and maintained by blood-meals at approximately five-day intervals first transmit the infection of *L. carinii* to white rat and cotton rat hosts on the occasion of the third meal, or the 15th day, after the mites' infecting meal. Transmission continues until the fifth meal on the 25th day, possibly with diminishing intensity, and it may occur until later than the seventh meal or the 36th day after the mites' infecting meal.

Of two cotton rats exposed to infection by 17-30 mites of a series in which 38 per cent. were positive, one rat which was exposed for 24 hours on the 15th day after the mites' infecting meal developed an infection averaging 275 mf./c.mm. of peripheral blood (range 90-670 mf./c.mm.) in 20 weekly counts; the other rat which was exposed for 24 hours on the 20th day after the mites' infecting meal developed an infection averaging, over 18 weekly counts, 97 mf./c.mm. of peripheral blood (range 40-190 mf./c.mm.). A third rat exposed to 3-13 mites of this series on the 25th day of their infection contained only one male *L. carinii*.

Cotton rats were found to be positive for the microfilariae of *L. carinii* in the peripheral blood-stream 51-54 days after a 24-hour period of exposure to infection by infected mites.

Non-infective forms of the worm with sickle-shaped tails occur on the ninth and tenth days of the worm's development, and the infective forms appear to be the active thread-like worms varying between 0.5 mm. and 1 mm. in length found from the 14th day to the 36th day after the mites' infecting meal.

The percentage infection-rates in two series of mites dissected before the first infective meal were as follows: 38 per cent. of 29 mites were positive for 1-5 worms after engorgement on a cotton rat showing 2,584 mf./c.mm. of peripheral blood; 21.8 per cent. of 39 mites were positive for 1-2 worms after engorgement on the same rat on a separate occasion when the blood count was 3,000 mf./c.mm. of peripheral blood.

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# FAILURE TO CONTROL *HYPODERMA* BY THE SPRAYING OF OVIPOSITION SITES ON CATTLE WITH 'GAMMEXANE'

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Flies belonging to the genus *Hypoderma* are among the most important arthropod parasites of cattle in this country. The proportion of damaged hides has been estimated at 35 per cent. of the total, and the annual financial loss, as a result of this damage, at £750,000. This is exclusive of losses due to partial condemnation at meat-inspection and of the not inconsiderable loss of milk due to the 'worry' caused by the peculiar oviposition habits of the fly. The economic importance of the pest was recognized by the Minister of Agriculture in 1936, and the Warble Fly Order of that year, and the Amending Order of 1942, made compulsory the treatment of warbled cattle by the application of derris in a standard dilution approved by the Minister. The restriction of derris supplies brought about by the recent war caused the suspension of the Order in 1942, and it has not, so far, been re-established.

The treatment with derris is directed against the larval stage of the parasite, and, although the damage to the hide occurs shortly after the larvae reach the back of the animal, the method is a most valuable one, in that it brings about the death of the grubs and the prevention of the next generation, and, if employed on a nation-wide scale, would undoubtedly result in a considerable reduction in, or complete elimination of, the warble-fly. The withdrawal of the Warble Fly Order interfered with the programme of national control, but at the same time it permitted a certain amount of research into the value of other insecticidal agents—notably of DDT and 'Gammexane'—which would otherwise not have been possible. Investigations with DDT, undertaken by Stewart (1944) and by Stewart (1946), suggested that these compounds were relatively ineffective as warble-fly larvicides. The possibility remained, however, that one might, by spraying the oviposition sites on the cattle, build up a sufficient concentration of the insecticide to kill either the adult fly, after it had alighted on the host, or the egg, or the first stage larva, before it had had time to penetrate the skin. The value of DDT in connection with such a method of control was investigated by Matthyse (1945). Only one application was made at the commencement of the warble-fly season, and this proved to be totally inadequate, despite the relatively high concentration (20 per cent.) of DDT which was used. It seemed not unlikely, however, that success might attend the repeated spraying of animals at short intervals, and it was to investigate the potentialities of this approach that the present work was undertaken.

The experiment was conducted on a self-contained Ayrshire herd owned by Messrs. Bibby and Sons Limited. The animals chosen were two-year-old heifers experiencing their first infestation with warble-fly larvae. For the purpose of the experiment the heifers were divided into three groups: group A, of 13 experimental animals; group B, a control group of 15 animals, untreated, but running with treated animals; and group C, again of 15 animals, also untreated, and running apart from treated animals. All the warbles present on the back of each heifer were counted, as far as possible every week, from the end of March, 1946, onwards, although in some of the animals which were not available at that time counts of warbles were made only from the end of April.

The number of warbles present in 1946 varied to a marked degree among individual animals and among those belonging to each group, even though the animals were of comparable age and had, presumably, been exposed to an equal risk of infestation. The maximal number of warbles in the various groups of animals ranged from 12 to 37 in group A and from 1 to 64 in group B. In both these groups the range 20-30 showed a tendency to occur with the greatest frequency, although there was a fair amount of 'scatter' on either side of this range. In group C, an almost equal number of animals fell within the ranges 1-10 and 10-20, the general level of infestation being somewhat lower in this group.

During 1946 the 13 animals of the experimental group were sprayed weekly, from the first week in May to the end of September, with an emulsion of 'Gammexane' in miscible oil and water, using a pressure-sprayer of the knapsack type. The miscible oil contained 5 per cent. weight volume of the active gamma isomer of benzene hexachloride (hereafter referred to as 'Gammexane'). Sixty c.cm. of the oil were made up to 3 litres with water, and the whole of this quantity (approximately 3 gm. of 'Gammexane') was deposited upon the animal. This quantity of solution had been found, by a process of trial and error, to give maximum wetting with a minimum of 'run-off.' The parts of the animal sprayed were those upon which the flies are known normally to oviposit, namely, the legs and belly, up to a line drawn half-way along the flank, the brisket, the escutcheon and the base of the tail. Assuming the area sprayed to be about 30 sq. ft., this would give a deposit-rate of approximately 100 mgm./sq. ft.

During the course of the experiment, tests were made at the end of one hour and of one week after treatment, to determine the length of time for which the insecticide persisted on the animals. The technique used was to expose house-flies to hair samples removed from the sprayed animals, control flies exposed to untreated hair being used for purposes of comparison. Such experiments revealed that 'Gammexane,' at the concentration used, failed to persist in the hair of the treated animals kept under field conditions, and that all trace of the insecticide, as measured by the above biological test, had disappeared at the end of one week after spraying. Later tests of this type showed that more than four times the amount of 'Gammexane' as that used in the present experiment, or a quantity greater than 12 gm. per animal per week, would be required if a sufficiently high residual effect were to be obtained. The use of such large quantities of 'Gammexane' would, of course, put the method outside the limits of practical economy.

In spite of growing conviction that the dosage of 'Gammexane' used was insufficient materially to affect the parasite, the spraying was continued until the end of September, 1946, when operations ceased until the following spring. Many animals calved during the

autumn and winter, and passed into the milking-herd ; others were sold ; so that a smaller number was available for observations during the early part of 1947.

As was expected, the warbles present on the backs of practically all the animals in 1947 showed a substantial reduction in number compared with the previous year. Counts of warbles were made on only three occasions during April and May. These observations showed that, of the eight animals remaining in the sprayed group (group A), six were affected with warbles ; of 13 animals in the control group B, which had been pastured with the sprayed animals in 1946, nine were warbled ; and of the 12 animals in group C, pastured apart from sprayed animals, four only were warbled. The frequency-distribution of the various numbers of warbles in the three groups is shown in the table below.

TABLE  
Showing the frequency-distribution of the number of warbles present among sprayed and unsprayed animals during the period April to May, 1947

Group	No. of animals	No. of warbles per animal						
		0	1-3	3-6	6-9	9-12	12-15	15-18
Group A (sprayed) ...	8	2	0	4	0	2	0	0
" B (unsprayed) ...	13	4	5	1	3	0	0	0
" C ( " ) ...	12	8	2	0	1	0	0	1

It is clear from the text and from the data given in the table that 'Gammexane,' when used under the conditions of this experiment, is without obvious action against the adult warble-fly, its egg, or the first stage larva.

It is not possible to define the cause of this failure, but it appears probable that, had the insecticide persisted on the hair and skin of the cattle in sufficient concentration for a period of a week, the result might quite well have been different. The rapid disappearance of DDT from the coat of the live animal has already been pointed out by Wells (1944), who found that the crystals deposited rapidly broke away from the hair. This observation has been partly substantiated by Vanderplank (1947), who sprayed oxen with DDT emulsions as a control-measure against *Glossina*. This author states that 'it appears that the loss of effectiveness on the living oxen is due to some action concerned with the living animal. It may be absorbed by the hairs or skin of the living animals or what is more likely is that it is rubbed off by the cattle rubbing up against one another, by friction with passing grass . . . and bushes, also when the animal lies down to rest.'

That a somewhat similar state of affairs occurs in the case of 'Gammexane' was shown in the course of the present experiments. It was noted that hair removed from an animal one hour after spraying and stored in the laboratory retained sufficient insecticide at the end of one week to kill house-flies exposed to it in one hour ; other samples of hair, taken at the same time but suspended for one week in wire containers in the open, gave a complete kill after three hours. On the other hand, samples removed from an animal one week after spraying were without lethal effect on the flies.

As a result of his experiments with DDT Wells suggested that some agent should be sought which, when mixed with the insecticide, would cause the crystals to adhere more firmly to the hair. The present extended observations show that this is equally true in the case of 'Gammexane.' The problem is an important one, for it would appear to

represent the chief limiting factor in the use of these insecticides upon living animals as a means of controlling parasitic arthropods.

### SUMMARY

1. An account is given of an attempt to control the warble-fly by the spraying of oviposition sites on cattle with 'Gammexane.'

2. Animals treated by weekly applications of 3 gm. of 'Gammexane' to the oviposition sites showed no diminution in the numbers of warbles in the subsequent season as compared with untreated controls.

3. By means of a simple biological test it was shown that 'Gammexane,' in the amount used in the experiment, failed to persist for one week on the hair of the live animal kept under field conditions. That this transient residual effect was connected in some way with the living animal was demonstrated by the fact that hair taken from cattle shortly after spraying, and kept in the open in wire containers for a period of one week, showed a marked residual effect.

4. Subsidiary experiments showed that more than four times the quantity of 'Gammexane' as that used in the experiment, i.e., more than 12 gm. per animal, would be required if a residual effect of one week's duration were to be obtained.

5. The authors' observations support those of other workers, and suggest that a search should be made for some agent which, when mixed with the insecticide, will prolong its residual effect on the hair and skin of animals.

ACKNOWLEDGEMENTS.—We should like to express our acknowledgements to Messrs. Bibby and Sons Limited and to their staff at the Home Farm where the experiment was conducted; also to Mr. G. H. Arthur, M.V.Sc., M.R.C.V.S., who took charge of the work for a short time during the authors' absence; and to our colleague, Miss Enid W. Roberts, B.Sc., for additional assistance.

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# HERMAPHRODITES OF *SCHISTOSOMA MANSONI*

BY

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In 1931, while dissecting guinea-pigs artificially infected with cercariae of *Schistosoma mansoni* in West Africa, I encountered several males which presented an organized but otherwise ill-defined mass of cells behind the testes, in a situation normally occupied by parenchymatous tissue. It was variable in size and shape, stained easily with alum carmine, and resembled a germ-gland. No further study was made at the time, and no similar observations were found in the literature.

Some years later, Professor R. M. Gordon, of the Liverpool School of Tropical Medicine, when visiting Hamburg showed me specimens of males of *S. mansoni* which, I believe, had been recovered from guinea-pigs and which showed the same feature. No satisfactory explanation of the significance of this group of cells was known to either of us. I was inclined to the belief that they might be supernumerary testes, but the frequency with which they occurred did not support this possibility.

In the years 1939-40, during the course of experimental studies on *S. mansoni*, I again encountered several male parasites with the same supernumerary organ, and I then undertook a thorough investigation of the matter, using both the newly obtained specimens and the old ones. The results were surprising, and may make an additional contribution to the interesting and intricate biology of sex of the genus *Schistosoma*.

## MATERIAL

The specimens about to be described were fixed in Bouin solution, stained in alum carmine and mounted whole in dammar resin. All the figures illustrating this paper are camera-lucida drawings of specimens thus treated.

The specimens of adult schistosomes were obtained by exposing guinea-pigs, hamsters, mice and rabbits to the cercariae of *S. mansoni* which had developed in *Planorbis* sp. Two of the guinea-pigs, two of the hamsters and several mice were infected with male schistosomes only, which were obtained by previously exposing the animals to cercariae produced by snails which had been exposed to a single miracidium each.

## DESCRIPTION OF THE RUDIMENTARY FEMALE REPRODUCTIVE ORGANS FOUND IN THE MALE

The structure in question is located in the median line of the parasite within an area limited in front by the group of testes, laterally by the intestinal branches, and behind by the union of these branches. In some males it occupies about the centre of this area, in others it is situated nearer to the testes or—more frequently—to the intestinal junction (fig. 1). In the latter case, its position in relation to the intestinal tract corresponds approximately to that of the ovary in the female. Sometimes, not one but two, or occasionally even more, of these organs were found in a single male, all set in line and irregularly interspaced.

The size of the organ varies considerably. The smallest examples are about the size of a testis and have a diameter of  $50\text{--}60\mu$ . The largest example measured  $362\mu$  in the longitudinal axis and  $138\mu$  in the maximum traverse diameter. The nearer the organ is situated to the posterior intestinal junction, the larger and more developed it generally is. Smaller structures are spherical, or nearly so (fig. 2, *b*). The longer ones are elongate, their long axis running parallel to that of the body, and they are marked by more or less

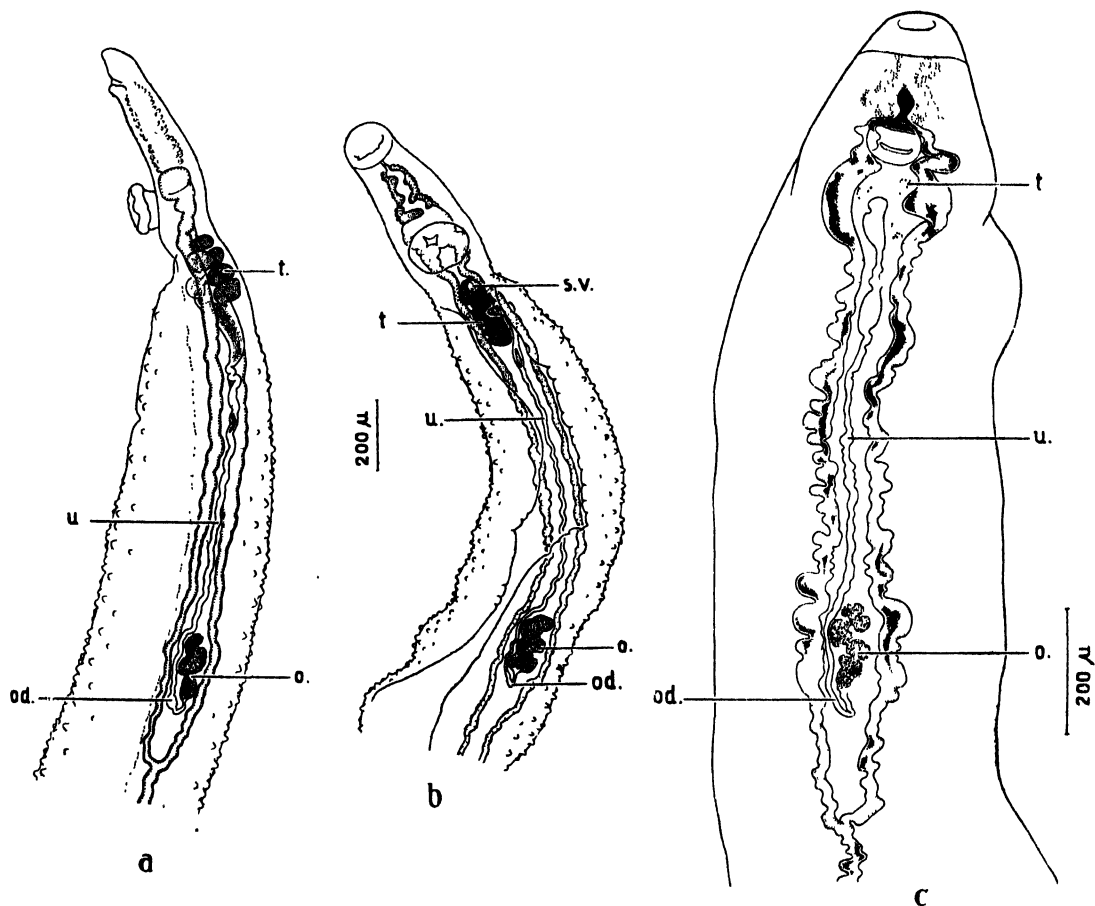


Fig. 1. Three secondary hermaphrodites of *S. mansoni*, showing the presence of ovaries and female genital ducts in addition to the testes. *a* and *b*.—Specimens from guinea-pigs; *c*.—Specimen from a rabbit (testes rudimentary). *t*.—Testes; *s.v.*—Seminal vesicle; *o*.—Ovary; *od*.—Oviduct; *u*.—Uterus.

regular protrusions and constrictions, thus presenting an undulating outline similar to that of the ovary of female schistosomes (fig. 2, *a*, and fig. 3).

The cell clumps are dark red, and contrast markedly with the surrounding pale parenchymatous tissue. The smaller clumps are generally of a uniform cellular texture similar to that of the testes or the anterior portion of the ovaries, and consist of ill-defined cells with nuclei rich in chromatin and with comparatively little protoplasm.



In the more extensive clumps, as well as in some of the smaller ones, a different type of cell occurs in addition to those described above. These cells are distinctly larger, with well-defined polygonal outlines, a broad darkly stained protoplasmic margin, and large nuclei poor in chromatin and provided with a conspicuous nucleolus. In every respect they resemble the fully developed egg-cells (oöcytes) of the ovary (figs. 2 and 3), which in female schistosomes occupy the posterior part of the ovary. We find the same arrangement in the supernumerary organ of the male, though there are instances in which these large polygonal cells fill the anterior region or are scattered irregularly. In a few

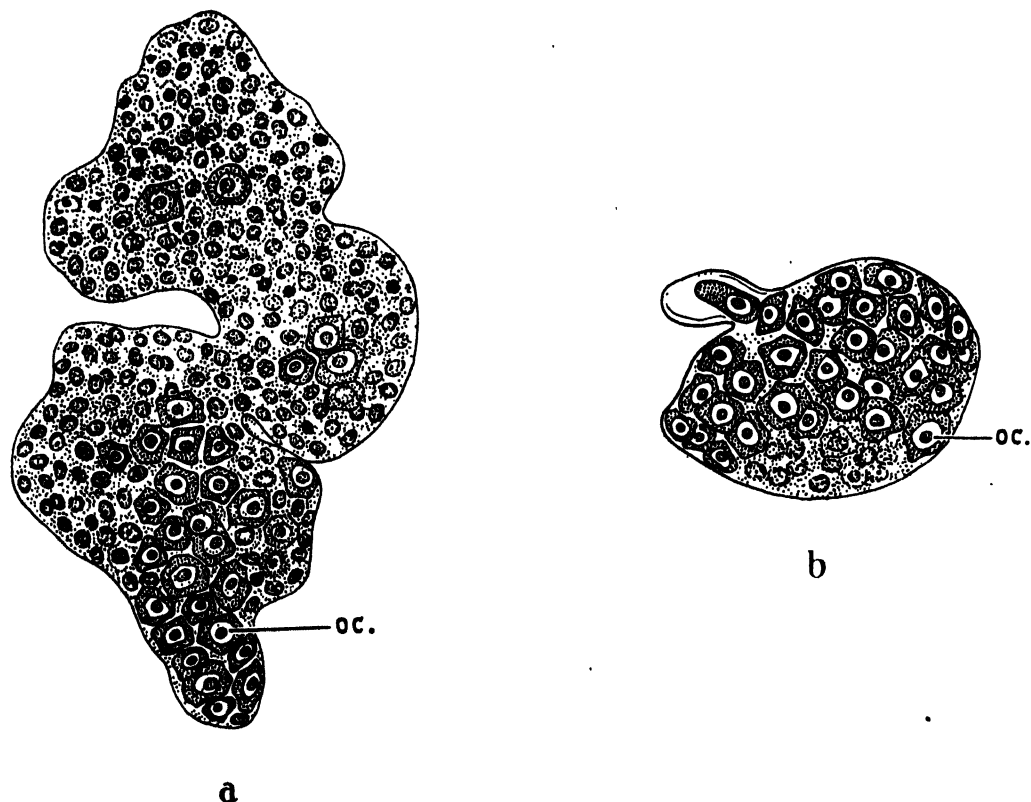


Fig. 2. Two ovaries of hermaphrodites of *S. mansoni* from guinea-pigs. Anterior pole uppermost. Scale as in fig. 3. oc.—Oöcytes.

specimens the whole organ is built up of this second type of cell. Except in two cases, which will be discussed later, no spermatozoa were seen in the organ.

When the supernumerary organ is well developed, a duct is sometimes visible in the space between the intestinal branches: it originates at the posterior pole of the organ, runs backward for a short distance, makes a complete turn and passes close to one side of the organ, and then follows the median line in a straight or slightly tortuous course until it reaches the group of testes (fig. 1). Whereas the anterior portion of the duct generally stands out clearly, the more delicate posterior part is often hardly discernible.

This canal takes exactly the same course as the oviduct and uterus in the female, the delicate posterior portion corresponding to the oviduct and the more solid anterior part to the uterus.

The position and shape of the whole structure suggest that we are dealing with a female organ. The absence of spermatozoa and the presence of typical egg-cells and, in some cases, of a canal corresponding to the female genital duct furnish additional and conclusive proof of the presence of an ovary in the male schistosomes. Specimens showing such a condition must, therefore, be regarded as hermaphrodites.

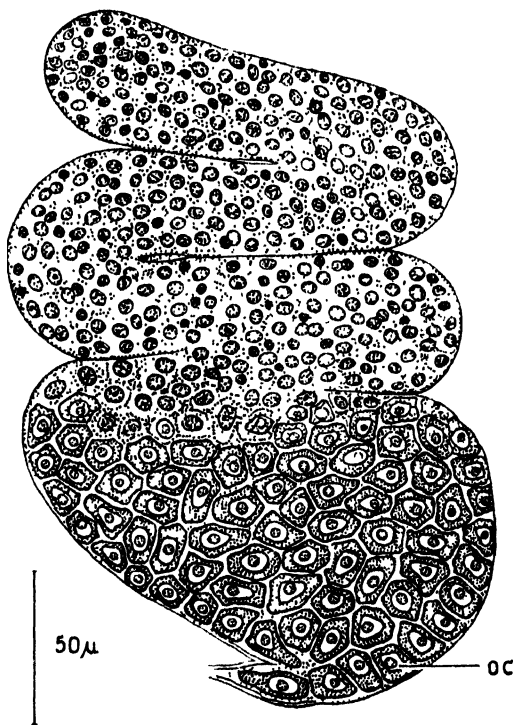


Fig. 3. Ovary of a mature female of *S. mansoni* from a guinea-pig. Anterior pole uppermost. oc.—Oocytes.

No oötype could be discerned in males provided with a female genital canal. The oviduct is empty, except in a single case in which it contained two well-stained egg-cells. The anterior portion of the uterus sometimes terminates in the neighbourhood of the posterior testes, but as a rule it proceeds a short way forward along one side of the testicular row, then turns over to the ventral side and ends in the mid-ventral line, just beneath the covering of the body at the level of the anterior testes. This terminal portion of the uterus is expanded and club-shaped (fig. 1). It ends blindly, which explains the accumulation of cellular material in its distal portion, and contains a colourless or yellowish mass which appears to consist of disintegrating egg-cells, the other portions of the uterus being empty. A female genital pore could never be made out with certainty, although in one specimen a short cord of tissue was seen connecting the conical end of the uterus

with the ventral skin close to the male genital opening, probably representing a rudimentary female genital pore.

In only 17 hermaphrodites of my collection is the ovary accompanied by a distinct female genital canal; 13 of these specimens were collected from guinea-pigs, three from a rabbit and one from a mouse. It is a peculiar fact and worth recording that, while 12 out of the 13 specimens from guinea-pigs were recovered from a few animals infected in West Africa in 1931, my numerous experiments performed later in Hamburg with the same host yielded only one such specimen. As a rule, neither uterus nor oviduct is recognizable in males with ovaries. In one specimen a solid cord of epithelial cells without a lumen takes the place of a tubular uterus. In other specimens one or more elongated or roundish hollow spaces lined with a thin membrane are to be seen in the mid-line between the intestinal branches, probably corresponding to sections of an incompletely developed uterus.

Some of the males recovered from guinea-pigs or other hosts show yet another morphological peculiarity which does not occur in normal males and of which no mention appears to be made in the literature. On both sides of the caecum small solid groups of cells are seen in varying numbers, their dark red-brown coloration contrasting distinctly with the parenchyma and resembling follicles of a gland. Fig. 4 shows a specimen containing a fair number of these small bodies. In other males I have found only a few such bodies, isolated here and there along the caecum, which I should certainly have overlooked had my attention not previously been drawn to them by their appearance in considerable numbers in other specimens. These gland-like structures are usually oval or spindle-shaped. Their long axis runs transversely to the body axis and measures from  $15\mu$  to  $45\mu$ . Small yellowish-brown droplets are to be seen in the protoplasm of the cells composing the follicles. These cells conform in every way—especially with regard to the above-mentioned inclusions—to those of the vitelline gland of the female worm. This conformity becomes particularly evident when preparations are examined of males and females fixed *in copula*, since in such cases it is possible to compare the vitelline gland of the female and the gland-like structure of the male within the same microscopic field (see fig. 5). In view of their cytological structure and of their arrangement along the caecum we have to interpret these follicles as parts of a rudimentary vitelline gland. Neither the excretory ducts of the follicles nor a main vitelline duct could be seen—a fact which points to the conclusion that production of ova is impossible in these hermaphroditic males.

In males which have developed an ovary or a vitelline gland or both, the testes, as a rule, are normally developed and function normally, as is shown by the spermatozoa which fill the seminal vesicles. Nevertheless, there are some specimens in which the female organs appear to have developed at the expense of the male glands, in that either the number of the testes is reduced or their tissues have not attained the stage of seminal production. And in extreme cases, observed especially in males recovered from rabbits, the testes, on superficial examination, seem to be totally missing, only a careful search revealing inconspicuous groups of cells corresponding to the very immature stages of testicular development (fig. 1, c).

Four exceptional cases are worth describing. In two males recovered from a hamster and a guinea-pig respectively, which at first sight had been considered 'pure' males, the unusual length of the row of testes attracted attention. Instead of the usual 6–8 testes,

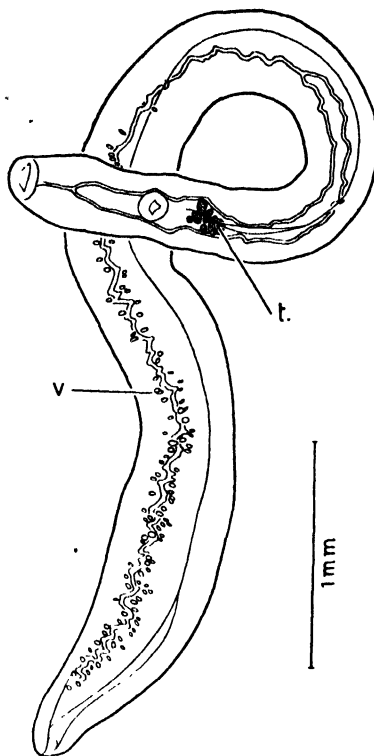


Fig. 4. Male of *S. mansoni* provided with a rudimentary vitelline gland; follicles arranged along the caecum. v.—Vitelline follicles.

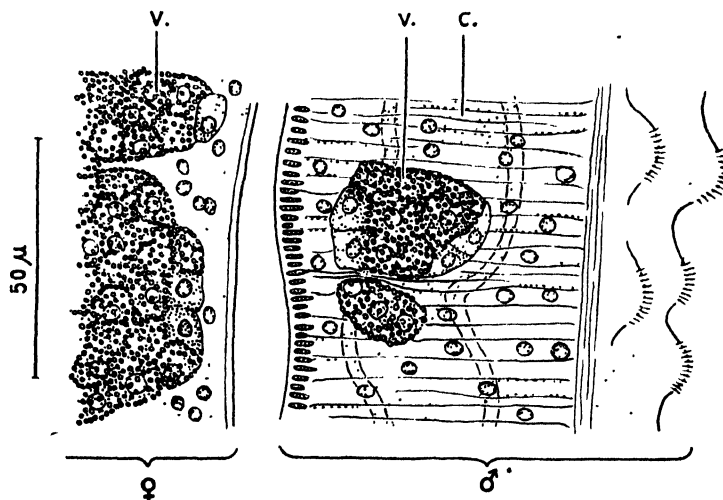


Fig. 5. A male and female *S. mansoni* taken in copula from a mouse; the portions illustrated show two vitelline follicles in the case of the male (lateral view, dorsum to the right), and in the case of the female a marginal portion of the vitelline gland. v.—Vitelline follicles; c.—Caecum.

there were 10 and 11 organs respectively, each closely following the other. Examination revealed that only eight and 10 organs respectively contained spermatozoa, the rest of the organs, situated at the posterior end of the row, containing unmistakable oöcytes. While the hermaphroditic forms described above always showed a clear separation of ovary and testes, the ovaries—two and one respectively—of these exceptional specimens were lying within the row of testes and had the spherical shape of the male organ.

The reverse condition occurred in a male recovered from a mouse. Here a supernumerary germ-gland was situated slightly in front of the junction of the intestinal branches—a position usually occupied by the ovary, which it resembled in its tortuous shape. However, since the gland contained numerous spermatozoa, it must be considered as a displaced testis.

The fourth exceptional case concerns a male with a well-developed ovary containing numerous typical oöcytes. At its posterior pole the egg-cells were surrounded by a number of minute darkly stained bodies indistinguishable from the spermatozoa present in the testes and seminal vesicle of the same male. If the interpretation of the dark granules as spermatozoa is true, we must suppose that the gland has produced female as well as male germ-cells. As the gland was not connected with a female genital duct, the spermatozoa could not have entered by this route.

The general shape of the hermaphrodite is that of the ordinary male (fig. 1). The flat body is comparatively broad, and forms a gynaecophoric canal with its concave ventral surface. The cuticle of the dorsum is tuberculated. There are no secondary sexual attributes peculiar to the female, nor could I find intermediary forms between male and female. So far, therefore, as the whole aspect of the body is concerned, the female characters of the hermaphrodite are strictly limited to the genital organs.

## DISCUSSION

It is commonly assumed that hermaphroditism is primary in trematodes, and that sex separation in schistosomes and related genera is a comparatively recent acquisition. Accordingly, the occurrence of hermaphroditic forms in *S. mansoni* must be regarded as an atavism. It is further to be assumed that the male of this species, of which the shape approximates to that of the average trematode—especially when the lateral parts of the body are unfolded—has preserved the anatomy of its ancestors more closely than has the female, the nematode-like figure of which appears to be a special adaptation to life within the gynaecophoric canal of the male. It is therefore quite probable that the hermaphrodites described and figured above give us a fairly true image of the hermaphrodite ancestors of the schistosome. The term 'atavism' illustrates only the phylogenetic aspect of the problem and certainly cannot completely explain the significance of hermaphroditism in *S. mansoni*. I have therefore tried to determine by experiment the conditions which influence the formation of these remarkable forms, and two factors deserve special attention.

The species of the host appears to play an important part. At first I had observed hermaphrodites among schistosomes recovered from guinea-pigs only. Later I found them also in hamsters and rabbits, but not in monkeys (*Cercopithecus* sp.) and seldom in mice. Perhaps it is the less suitable host that favours a certain lability of sex-determination, for guinea-pigs and, to a still greater extent, rabbits cannot be considered

'good' hosts like monkeys and mice. In guinea-pigs and rabbits egg-production of *S. mansoni* is limited; many ova degenerate in the tissues before reaching the miracidium stage, and none are evacuated with the faeces. Furthermore—at least in the rabbit—the growth of the worm is impaired. In monkeys and mice, on the other hand, development and evacuation of eggs and the growth of the worm are normal. The hypothesis, however, is inconsistent with the fact that in the hamster (*Cricetus cricetus*), which is a suitable host in every respect, hermaphrodites occur in large numbers under certain conditions to be discussed later, while, under similar conditions in the mouse, which is also a 'good' host, they do not occur. Although it remains obscure how the host might cause sex lability in the parasite, we have to accept the fact that some species of hosts favour the development of hermaphrodites of *S. mansoni* while others do not.

When guinea-pigs harbouring hermaphrodites were dissected, the scarcity or total absence of females attracted attention and suggested the possibility that the absence of a female partner might be another factor in causing the development of an ovary in the male.

In order to test this theory I exposed two guinea-pigs to male *mansoni* cercariae only, two more guinea-pigs simultaneously infected with both sexes of cercariae from seven *Planorbis* being used for comparison. The four guinea-pigs were killed and examined 3–4½ months after exposure to infection. The two guinea-pigs infected with both sexes yielded 70 male worms, of which only three, or 4·3 per cent., were provided with a female germ-gland. Considerably more females than male worms were found in these animals—a most unusual finding in guinea-pigs—and, as a result of this female surplus, practically all the males were found *in copula* and were therefore especially suitable for comparison. From the pair of animals infected with male cercariae only, 83 males were recovered, 36, or 43·4 per cent., of which contained an ovary. Hence the percentage of males which developed into hermaphrodites was 10 times greater amongst those which had developed unmated than amongst those which had mated with females.

The results of nine further experiments performed on guinea-pigs for other purposes may also be mentioned here. Each animal had been exposed to cercariae of many heavily infected snails, and thus a more or less equal number of male and female cercariae may be presumed. Nevertheless, when the animals were examined at autopsy, the males always outnumbered the females. These results in general confirm those of Gordon, Davey and Peaston (1934), who found that, with the same parasite and host, in some animals males only developed, while in others, which harboured both sexes, the males were more numerous than the females. The two guinea-pigs previously referred to, which permitted the development of more females than males, must therefore be regarded as exceptional. Of 237 male worms recovered from the nine guinea-pigs of my experiment, 53, or 22·4 per cent., contained an ovary. This intermediate figure between the extremes of 43·4 per cent. and 4·3 per cent. mentioned above was to be expected, since some, though not all, of the males had a partner for copulation.

The experiments performed on guinea-pigs were then repeated on hamsters (*Cricetus cricetus*). Two of these animals were infected with male cercariae only, which originated from snails each exposed to a single miracidium. When dissected four months later, the two hamsters yielded a total of 92 male worms, of which 37, or 40·2 per cent., possessed an ovary. Four more hamsters were exposed to cercariae of both sexes. A few months later they harboured about as many males as females. In three of these

animals 37 males were found, all without an ovary; in the fourth, one worm out of 14 was found with a female genital gland; in other words, only one out of a total of 51 males, or about 2 per cent., had reached the hermaphrodite stage. In this host, therefore, the increase of hermaphrodites caused by the absence of females was twentyfold.

Mice used as hosts gave quite different results. In searching for hermaphroditic forms I examined many specimens of male *S. mansoni* recovered in previous experiments on mice. The material consisted of 247 male worms taken from mice which had been exposed exclusively to male cercariae, and 56 males from bisexually infected mice. All of the latter group had been found *in copula* when removed from the blood-vessels. All worms of both groups had attained their full size and were sexually mature. Three males among the unpaired of the first series and one of the paired males showed an ovary. The percentage of hermaphrodites is therefore 1.2 in the first group and 1.9 in the second. This result indicates that, contrary to my experiments on guinea-pigs and hamsters, in mice hermaphroditism is not promoted by the absence of females.

Simultaneously with the two guinea-pigs already referred to, which had been exposed to male cercariae originating from a single miracidium, a mouse was infected with cercariae from the same source. Three months later 37 males were recovered from this animal: all were without an ovary, although in the two guinea-pigs almost half the worms were hermaphrodites. It follows from this experiment that, although all the worms had originated from the same egg-cell, and consequently had the same genotype, they had developed partly into 'pure' males, partly into hermaphrodites, a high percentage of the hermaphrodites occurring in the guinea-pigs and none but 'pure' males in the mouse. This experiment not only confirms the statement made above that mice, used as hosts, do not favour the formation of ovaries in the males of *S. mansoni*, but also furnishes conclusive proof that hermaphroditism of this species does not depend on the genotype, and that the hermaphrodites found are genetically males. They are therefore classified as secondary hermaphrodites (Hartmann, 1943), a condition very similar to that observed in certain amphibians, especially toads, of which a proportion of the adult males develop ovaries and oviducts in addition to the male sexual organs (Fuhrmann, 1913).

The transformation of males of *S. mansoni* into secondary hermaphrodites evidently depends on environmental influences. My experiments have revealed two factors—the influence of the host species and the absence of female partners. The second factor still needs a more detailed consideration. What is the significance of the fact that the lack of a female partner promotes the appearance of female sexual organs in the male? And is there a causal explanation of the phenomenon?

From the teleological point of view, this remarkable fact might be interpreted as an attempt by the parasite to produce a substitute for the missing female in the interest of the preservation of the species—an attempt which, however, does not fully attain its aim, i.e., propagation. But the author feels that by such reasoning he would be going outside the bounds of strict natural science, and that an attempt to find a causal explanation would be more justified.

When dissecting a rabbit which had previously been exposed to male cercariae only, Giovannola (1936) observed three pairs of *S. mansoni in copula*, each consisting of two males. I also repeatedly encountered such homosexual couples at the autopsy of animals experimentally infected with males of *S. mansoni* or *S. japonicum*. As a rule, a large male had enclosed a small one in its gynaecophoric canal. The partners of such couples separate

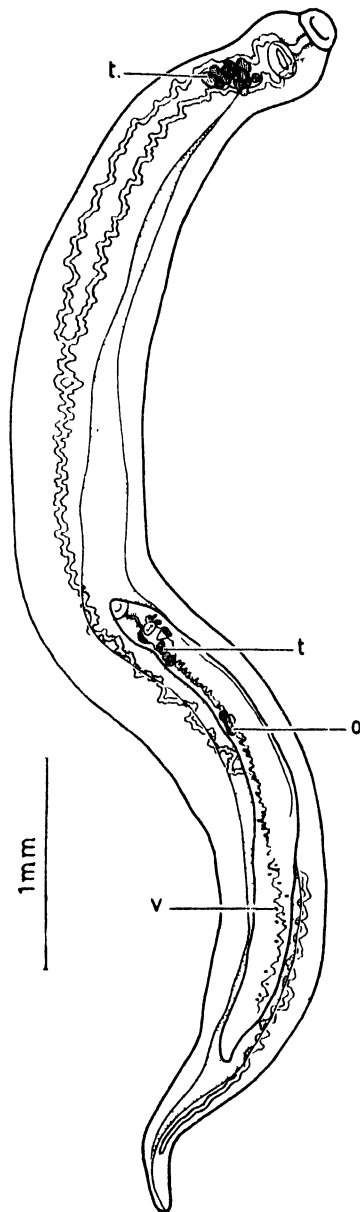


Fig. 6. A pair of *S. mansoni* taken from a hamster infected with male cercariae only; the large partner is a 'pure' male, the small embraced male is hermaphroditic. t.—Testes; o.—Ovary; v.—Vitelline follicles.



more readily when handled and fixed than those of ordinary heterosexual pairs. It can be assumed, therefore, that the number of homosexual couples is higher in the living host than after the transference of the worms into normal saline for examination. Most probably the loose contact of male partners results in frequent separations and reunions within the blood-vessels of the living host.

My collection contains two examples of homosexual couples of *S. mansoni* from a hamster, fixed and stained *in copula*. The large worms, which play the part of the male partner, contain only male genital organs, while the small ones, which are enclosed in the canal of their partners, are hermaphroditic. One pair is shown in fig. 6. This observation suggests the tentative explanation that it is residence in the gynaecophoric canal which converts a male into a hermaphrodite. The fact that most hermaphrodites are found unpaired does not disprove this supposition, in view of the instability of homosexual couples. On the other hand, the presence of both sexes, even when females are in excess, does not exclude the possibility that occasionally two males may unite for some time and an ovary be developed in one of them. Unmated female schistosomes are known to remain small and sexually immature. It is residence in the gynaecophoric canal that brings the rudimentary female genital organ to full development (Vogel, 1941). Presumably the identical stimulus in the case of homosexual couples incites the development of a latent female genital 'anlage'\* in the embraced male, for it may be assumed that schistosomes, like all sexually differentiated organisms, are potentially bisexual, and that in a worm of one sex a dormant and indiscernible 'anlage' of the organs of the other sex is present.

We have not yet discussed whether the formation of rudimentary vitelline glands in the male depends on the same factors as the development of an ovary. I have recovered males with vitelline follicles from guinea-pigs, hamsters, mice and a monkey. Males of sufficient maturity taken from rabbits were studied only in small numbers, and no vitelline follicles were seen. Perhaps, if a larger number had been examined from the rabbit material, traces of the gland would have been revealed. Vitelline glands were found most frequently and most highly developed in males from guinea-pigs. It is a peculiar fact that in some guinea-pigs males with comparatively well-developed vitelline glands were found, while in others, under similar conditions, only a few follicles had developed. Similar observations were also made on other hosts, and males with fairly well-developed vitelline glands were recovered from mice, though less frequently than from guinea-pigs. In males taken from hamsters and from a monkey (*Cercopithecus* sp.) vitelline follicles were always scarce. Ovaries and vitelline glands seemed to occur independently from each other, for many males with comparatively well-developed vitelline glands contained no ovary, and in other males with an ovary vitelline follicles were scanty or missing. Comparing males which had developed with and without females, I have seen specimens with vitelline glands in both groups and in similar numbers. I also possess several preparations of heterosexual couples *in copula* with a male showing typical vitelline follicles. The lack of a female partner, therefore, appears to be without definite influence on the occurrence of this gland. It must be mentioned, however, that males with especially well-developed vitelline glands were found in animals harbouring male parasites predominantly or to the exclusion of females.

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\*Used in the sense of a genital primordium.

In searching for sexual abnormalities, I examined many specimens of *S. japonicum* recovered from eight different species of host and including many males which had reached maturity in the absence of females. Several of these males showed one supernumerary isolated testis behind the regular row of seven testes, and in one female a small additional ovary, not connected with a genital duct, was found in front of its normal germ-gland. Neither ovaries nor vitelline glands were found in any of the males. A study of 96 males of *S. haematobium* taken from mice, hamsters and monkeys, which also had partly developed in the absence of females, had the same negative result. The disposition to produce female genital organs in the male schistosome seems, therefore, to be peculiar to *mansoni*.

### SUMMARY

Secondary hermaphrodites of *Schistosoma mansoni* are described. They show the ordinary characters of males but are provided with an ovary, which, when well developed, contains typical egg-cells. The ovary is situated between the group of testes and the posterior intestinal junction. In some specimens an oviduct and uterus were found in addition to the ovary, but a female genital pore could never be made out with certainty.

Genetically these worms are males. Their transformation into secondary hermaphrodites is favoured by certain species of hosts (guinea-pigs, hamsters, rabbits) and occurs most frequently in the absence of a female partner. Thus guinea-pigs and hamsters infected with male schistosomes only always harboured a higher proportion of hermaphroditic males—10 times in the case of guinea-pigs and 20 times in the case of hamsters—than did similar animals infected with the normal proportion of male and female worms. Males *in copula* are described, the small embraced partner being a hermaphrodite and the embracing worm a normal male. The possibility is discussed that residence in the gynaecophoric canal, which is essential to sexual maturation of the female, may likewise stimulate the development of rudimentary female characters in the male.

Phylogenetically, hermaphroditism in schistosomes can be considered as an atavism. By teleological reasoning it might be interpreted as an attempt by the solitary male to compensate for the absence of a female partner.

The sexual abnormality appears to be peculiar to *S. mansoni*, since, under similar conditions, no hermaphrodites were found in *S. japonicum* or *S. haematobium*.

Rudimentary vitelline glands are also described in males of *S. mansoni*. They are situated at both sides of the caecum and occur regardless of ovary development.

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# ACQUIRED PALUDRINE-RESISTANCE IN *PLASMODIUM GALLINACEUM*

## I.—DEVELOPMENT OF RESISTANCE TO PALUDRINE AND FAILURE TO DEVELOP RESISTANCE TO CERTAIN OTHER ANTIMALARIALS

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### INTRODUCTION

In a preliminary note we have reported that a high degree of resistance to paludrine may develop in *Plasmodium gallinaceum* as a result of non-curative treatment in serially subinoculated chicks, and that this resistance is retained on passage through *Aedes aegypti* (Williamson, Bertram and Lourie, 1947). A similar preliminary announcement has been made, simultaneously with our own, by Bishop and Birkett (1947). Further details and discussion of some of our own observations are here presented, but particulars in regard to the retention of paludrine-resistance on passage through mosquitoes will be reserved for a later paper in this series.

The development of acquired drug-resistance in malaria parasites has often been postulated in the past, particularly during the first world war, when it was frequently invoked in order to account for the occurrence of so-called quinine-resistant malaria. Alternative, and at least equally plausible, explanations were, however, usually available, and acquired quinine-resistance in malaria parasites has never been unequivocally demonstrated. Probably the most intensive attempt to produce quinine-resistance was that of Lourie (1935), who treated *P. cathemerium* infections, in serial passage through canaries, with 1 mgm. quinine hydrochloride intraperitoneally every single day for as long as 511 days (one year and five months); similar attempts to produce resistance to plasmochin (pamaquin), by administration of 0.01 mgm. every day for 9½ months, were equally unsuccessful. Fulton (1942) also failed to produce resistance to plasmochin in *P. gallinaceum* in chickens, as a result of intermittent short courses of treatment over a period of 15½ months. He suggested that the explanation might lie in the fact that the exo-erythrocytic forms, demonstrated by James and Tate in 1937, do not respond to the treatment employed, and that they therefore survive treatment as a source of further drug-sensitive erythrocytic forms. It now seems clear, however, that pamaquin does exercise some effect on the exo-erythrocytic forms of *P. cathemerium* and *P. gallinaceum* (Kikuth and Mudrow, 1939; Coggeshall *et al.*, 1944; Davey, 1946b), and indeed Bishop

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and Birkett (1947) have now been able to produce pamaquin-resistance in *P. gallinaceum*. However, as shown by the experiments with sulphadiazine described below, the existence of drug-sensitive exo-erythrocytic stages in the life-cycle of a species of malaria parasite does not necessarily predicate a potentiality towards the development of resistance to the drug concerned. Prior to the present studies and to those of Bishop and Birkett the only incontrovertible demonstration of acquired drug-resistance in malaria parasites was that of Fulton and Yorke (1941, 1943), who produced pamaquin-resistance in *P. knowlesi* infections in monkeys, in which exo-erythrocytic forms have not yet been demonstrated, and in which it is conceivable, therefore, that the development of resistance may have been encouraged by the absence of any exo-erythrocytic source of drug-sensitive parasites.

The present investigation was started in February, 1944, before paludrine had been discovered, and at a time when it was of compelling importance to establish the suitability of mepacrine in place of quinine for the large-scale control of malaria by armies in the field. Quinine had, for practical purposes, already been adequately explored in regard to its potentialities for inducing drug-resistance, and it appeared to be remarkably free from this undesirable quality. Stocks of quinine were, however, highly precarious and were rapidly and overwhelmingly replaced by mepacrine, but no sustained investigation seemed yet to have been made of the possibilities of acquired mepacrine-resistance. It seemed important, therefore, that such an investigation should be undertaken, and we naturally chose, for this purpose, the cheapest and most convenient laboratory-infection available, *P. gallinaceum* in chickens.

At the same time the question was taken up of acquired resistance to a number of other antimalarials in *P. gallinaceum*. Quinine was again selected for examination in this respect, since it had not yet been explored in the case of *P. gallinaceum*, and it was thought desirable to establish one or other of the two possible eventualities, i.e., either that the inability to develop quinine-resistance is a property of yet another species of *Plasmodium*, or that it is a species-specific property. A suggestion of some such specificity seemed already to have been provided by pamaquin, to which, as mentioned above, resistance had been developed in *P. knowlesi* (Fulton and Yorke, 1941) but not in *P. cathemerium* (Lourie, 1935) or in *P. gallinaceum* (Fulton, 1942). Sulphadiazine seemed a suitable object for similar studies, because its constitution differed radically from that of other antimalarials, and because of the evidence that its mode of antimalarial action likewise differed from that of the other compounds (Marshall *et al.*, 1942). The case for including sulphadiazine in this investigation was further strengthened by the knowledge that it exercises some activity against exo-erythrocytic forms of *P. gallinaceum* (Coatney and Cooper, 1944; Coggeshall *et al.*, 1944), and it thereby provided the opportunity for testing the possibility that reactivity of the exo-erythrocytic forms of a parasite might be conducive to the acquirement of resistance towards the drug concerned. With the development of the novel series of pyrimidine antimalarials by Curd, Davey and Rose (1945a) it became advisable to include also at least one member of this series in the investigation, and the obvious choice at the time (early 1944) was 3349,\* the first compound of the series shown to be effective against human malaria (Adams and Sanderson, 1945).

Our strain of *P. gallinaceum* was accordingly passaged through chickens in four series, respectively subjected to intensive treatment with each of these four compounds. After

\* 2-*p*-Chlorophenylguanidino-4- $\beta$ -diethylaminoethylamino-6-methylpyrimidine dihydrochloride.

nearly 2½ years of such treatment it was clear that no resistance had yet developed to any of the four compounds, and it was thought advisable to examine paludrine in the same way, even if only for a relatively short time, before terminating the entire investigation. In 3-4 months the capacity for *P. gallinaceum* to acquire a high degree of paludrine-resistance was revealed.

#### ATTEMPTS TO PRODUCE RESISTANCE TO MEPACRINE, QUININE, SULPHADIAZINE AND 3349 RESPECTIVELY

Chickens of various breeds, mainly Rhode Island Red or Light Sussex, and about 6-9 weeks old, were used for the long series of infections and treatments with each of the four compounds of this paragraph-heading. The strain was usually passaged intravenously, but sometimes intramuscularly, from one bird which had already been treated to another which was to be similarly treated, almost invariably without any intervening untreated passages. The drug-treatments were administered by stomach-tube, and the routine procedure was to start the dosage as soon as parasites were easily to be found on microscope examination. This varied widely, from two or three days to about eight days after inoculation, the variations depending, of course, on the amount of blood, route of inoculation, and number of parasites injected. No attempt was made to inoculate a standard number of parasites; indeed it would not have been easy to maintain such a standard regularly, since it was not infrequently necessary to subinoculate when there were extremely few parasites present, and when it would consequently have been difficult to make an accurate parasite count. Treatments were administered once daily on consecutive days (except Sundays) for two weeks, i.e., for 12 doses per passage, unless the bird died within that period, as was very often the case. For each drug a standard size of dose per day was employed, as follows (per 50 gm. body-weight, in solution to give 0.5 c.cm. per 50 gm. body-weight): mepacrine methanesulphonate, 10.0 mgm.; quinine hydrochloride, 7.5 mgm.; sulphadiazine, 25.0 mgm.; 3349, 10.0 mgm. The history of the treatments in these four lines of serially infected chickens (series 1-4) is shown in Table 1. The last column of the table, listing the average number of doses per passage, indicates that birds seldom survived the full 12-dose course of mepacrine, quinine or 3349, but that they usually withstood the full course of sulphadiazine. The birds which died during treatment with mepacrine, quinine or 3349 were found, almost invariably, to be heavily infected with exo-erythrocytic forms. Probably this condition supplemented the undoubtedly severe toxic effects of the high dosages of these three drugs as the cause of deaths, but no attempt was made to determine whether the same treatment-courses in uninfected chicks would have produced similar mortality-rates. In the occasional birds which died during treatment with sulphadiazine, exo-erythrocytic forms could generally not be found, thus supporting the findings of other workers that sulphadiazine, unlike mepacrine, quinine and 3349, is conspicuously active against this usually intractable stage of the parasite. The numbers of birds used and the doses given in the course of this investigation were actually considerably more than is shown in the table. In fact, at least two birds were inoculated at each passage, in order to ensure that the strain would not be lost by an early accidental death, and treatments were administered to both these birds, only one of which was, however, subsequently used in order to pass the strain forward at the next passage. The table shows, for each of the four drug-treated strains,

TABLE I

History of serial infections of *P. gallinaceum* in chicks treated with mepacrine, quinine, sulphadiazine and 3349 respectively

Series	Treated with	Duration of treatment		Dosage (bv stomach-tube)			
		Total (months)	No. of consecutive passages	Size of each dose (mgm. per 50 gm.)	Total no. of doses	Aggregate dosage (mgm. per 50 gm.)	Average no. of doses per passage
1	Mepacrine	27	69	10.0	438	4,380	6.3
2	Quinine	29	73	7.5	516	3,870	7.1
3	Sulphadiazine	27	44	25.0	500	12,500	11.4
4	3349	28	73	10.0	483	4,830	6.6

TABLE II

Comparison between response of the parent strain and of strains 1-4 (see Table I) to mepacrine, quinine, sulphadiazine and 3349 respectively

Drug tested	Experiment	Strain		Results at			M.E.D (mgm.)
		No.	Previously treated with	4 mgm	2 mgm	1 mgm.	
Mepacrine	A	P* 1	— Mepacrine	3/366	3/445 42/366	406/445 288/366	2 2-4
	B	P 1	— Mepacrine	1/344	147/410 59/344	356/410 323/344	4 2-4
Quinine	A	P 2	— Quinine	13/414	183/405 103/414	387/405 308/414	4 4
	B	P 2	— Quinine	1/381	22/374 13/381	295/374 171/381	2 2
Sulphadiazine	A	P 3	— Sulphadiazine	19/401	17/404 34/401	55/404 172/401	2 2-4
	B	P 3	— Sulphadiazine	17/387 †27/363	248/387 25/363	257/387 52/363	4 4
3349	A	P 4	— 3349	17/386	178/430 33/386	401/430 371/386	4 4
	B	P 4	— 3349	7/388	304/428 174/388	391/428 382/388	4 4

\*P = Parent strain.

†Result at 8 mgm. : 9/363.

The results are given as in the contribution by Davey (1946a). Treatments were by stomach-tube at above dosages per 50 gm. body-weight, twice daily for 3½ days, starting on the day of inoculation. The numerator is the average count of parasitized cells per 500 R.B.C.s in 6 treated chicks on the 5th day of infection, and the denominator the average count on the same day in 6 untreated control chicks.

the number of passages and treatments experienced only in the direct line of descent from the beginning to the end of the  $2\frac{1}{4}$ - $2\frac{1}{2}$ -year period.

Each of the four strains appeared to respond to the respective treatments equally well at the end as at the beginning of the  $2\frac{1}{4}$ - $2\frac{1}{2}$ -year period, that is, an acute parasitaemia was always prevented during the course of daily treatment, the parasite count seldom rising above 3 or 4 per 100 microscope fields. However, in order to obtain a more exact quantitative comparison between each of the drug-treated strains on the one hand, and the parent strain (previously untreated by any drug) on the other, a series of determinations of drug-activity were carried out according to the technique described by Davey (1946*a*). This is an elaboration of the method previously used in canaries by Lourie (1934, 1935), depending as it does upon (i) the inoculation of parasitized blood intravenously so that acute infections are produced with minimal prepatent periods, and (ii) the institution of daily drug-treatment immediately after inoculation, with the observation of the effect of such treatment on the parasite-number count at the peak of the time-parasite curve. Davey's technique was followed in all details, for particulars of which the reader is referred to his very serviceable original account. Table II shows the results of observations obtained in a number of experiments using this technique. In each experiment two strains were tested simultaneously, namely, the parent and one of the previously drug-treated strains, and, since the minimum effective doses proved to be the same, within experimental error, in the two strains of each pair tested, it is clear that no drug-resistance had been acquired as a result of the several years' treatment with the respective compounds used.

#### DEVELOPMENT OF RESISTANCE TO PALUDRINE

In attempting to produce resistance to paludrine, the methods adopted were, in general principle, the same as those used in attempts to produce resistance to the other drugs, but there were certain differences of detail.

Only chicks which were six days old (on the first day of treatment) were used throughout for the serial passages and treatments. Two separate series were maintained, one (series 5) in which the infections were treated with doses of the order of the minimum effective for the normal strain, and the other (series 6) in which the doses were of the order of the maximum tolerated by young chicks. The object of maintaining two series, with widely different sizes of dosage, was that it was thought conceivable that paludrine-resistant parasites (if these were to arise at all) might stem from exo-erythrocytic rather than from blood forms. In this event resistance might take a very long time to develop (or might not develop at all) after maximal dosages which perhaps eliminate, or prevent the development of, exo-erythrocytic parasites under the conditions of our experiments (Davey, 1946*b*), whereas it might arise quite readily on a minimal dosage schedule, which influences the exo-erythrocytic forms whilst certainly not eliminating them.

The details of inoculations and treatments of the serial infections conformed to specifications of Davey's assay-technique, that is, birds were inoculated intravenously with approximately  $50 \times 10^6$  parasitized red cells, and the first treatment-dose (0.25 mgm. per 50 gm. weight for series 5, and 5 mgm. or 2.5 mgm. for series 6) was given 5-6 hours later, and then repeated twice daily, at about 9.30 a.m. and 4.30 p.m. for the next three days. Record was then made of the number of parasitized cells per 500 R.B.C.s on the following day, i.e., the fifth day of infection (when the count in untreated chicks, similarly inoculated, usually reaches its peak). Infections which had been thus treated then served,

after some days, as the source of parasitized blood for the infections of the next passage in the series. Usually an infection of this next passage was then treated, in the same way as its predecessor, but not uncommonly there were one or more intervening untreated passages between those treated as described. This technique was adhered to throughout the passages and treatments of these two series, in order that it might be possible, with some degree of accuracy, to note the actual time when resistance might appear. The treatment of each infection therefore served not only to provide for further contact of the strain with paludrine, but also as a test for the possible acquirement of resistance

TABLE III  
Development of paludrine-resistance in *P. gallinaceum* in the course of serial passage through chicks (series 5) treated with the minimum effective dose of paludrine

Passage no.	Dosage mgm./50 gm. ( $\times 7$ in $3\frac{1}{2}$ days)	Week of experiment	Parasitized cells per 500 R.B.C.s on 5th day of infection*
1	0.25	1	5. 40. 4
2	0.25	2	1. 1. 48
3	—		
4	0.25	4	2. 5. 56
5	—		
6	0.25	6	0. 1. 10
7	—		
8	0.25	8	0. 0. 4
9	0.25	9	0. 6. 15
10	0.25†	10	14. 46
11	0.25	11	2. 2. 6
12	0.25	13	61. 108. 142
13	—		
14	0.25	14	377. 485
15	—		
16	0.25	15	9. 73. 95
17	0.25	16	380. 430
18	—		
19	0.25	17	380. 400
20-22	—		
23	2.5	19	407. 430. 432
24	—		
25	2.5	20	370. 400. 428
26	—		
27	0.25	21	395. 427. 433
28	—		
29	0.25	22	385. 477
30	—		
31	0.25	23	410. 417. 437

\*Respective counts for the 2 or 3 infections of each passage.

†5 doses instead of 7.

consequent on the treatments immediately preceding. As in series 1-4, treated by compounds other than paludrine, two or three birds were infected and treated at each passage, as a precaution against loss of the strain through an early accidental death, but only one of these infections was used for passing the strain forward at the next passage. The results are set forth in Tables III and IV, which refer not only to infections in the line of direct descent, from the beginning to the end of the respective periods of 23 and 17 weeks, but also to the collateral infections, not used for passing the strain forward. The development of resistance, indicated by the parasite counts of the treated birds on the



day after the end of the treatment courses (the 5th day of infection), is shown in column 4 of these tables.

It may be seen (Table III) that in series 5, treated by the smaller dosages, the 5th-day parasite counts generally remained very low, with a few aberrations, up to the 11th week, by which time the strain had been passed through eight treated infections involving 54 doses of 0.25 mgm. in about  $2\frac{1}{2}$  months. Thereafter, resistance appeared to have become well established, and there was little room for doubt on this score after the 15th week, i.e., after 11 treated infections, involving 75 doses in about  $3\frac{1}{2}$  months. After two further courses of treatment with the customary small doses, the infections were (at the 19th week) inadvertently treated with a course of 2.5 instead of 0.25 mgm. per dose. They proved to be fully resistant to this, about 10 times the normal minimum effective dosage.

TABLE IV

Development of paludrine-resistance in *P. gallinaceum* in the course of serial passage through chicks (series 6) treated with maximal doses of paludrine

Passage no.	Dosage mgm./50 gm. ( $\times 7$ in $3\frac{1}{2}$ days)	Week of experiment	Parasitized cells per 500 R.B.C.s on 5th day of infection*
1	5	1	0. 0. 7
2	5	3	0. 1
3	5 or 2.5†	5	1
4-5	—	—	—
6	5‡	9	—
7-12	—	—	—
13	0.25	13	0. 0. 0
14	2.5	14	295. 360. 398
15	—	—	—
16	2.5	15	410. 440. 442
17	—	—	—
18	2.5	16	335. 337. 390
19	—	—	—
20	2.5	17	380. 410

\*Respective counts for the 1, 2 or 3 infections of each passage.

†4 doses of 5 mgm. followed by 3 doses of 2.5 mgm.

‡4 doses instead of 7.

In series 6 (see Table IV), treated with maximal doses, 5 mgm. per 50 gm. twice daily proved to be highly toxic. Of 12 chicks thus treated, four died within  $3\frac{1}{2}$  days, and in later treatments the individual dose was therefore reduced to 2.5 mgm. At the 13th week the infections were, also inadvertently, treated by twice-daily doses of the minimum curative dose, 0.25 mgm. instead of 2.5 mgm. It proved to be a fortunate mischance that this unintended dosage was given, since it showed that the strain had, at this stage, still retained unimpaired its pristine sensitivity to paludrine. The next passage was treated by a course of 2.5 mgm. twice daily for  $3\frac{1}{2}$  days, and proved to be completely resistant to that dosage. This high degree of resistance, at least a tenfold increase, therefore appeared quite suddenly after the strain had been passaged through five treated infections, four of which had received maximal doses, and one the minimum effective dose. The emergence of acquired resistance in these experiments seems therefore to be

attributable to the appearance, and then presumably the selection, of a mutant, differing very widely from the normal, rather than to the gradual training of parasites, by some unknown mechanism, to slowly increasing degrees of resistance.

Both the paludrine-resistant strains, 5 and 6, were treated still further with paludrine, in subsequent passages, after resistance had already come clearly into evidence. As shown in Table III, strain 5 was passaged, during 23 weeks, through a total of 31 serial infections, 16 of which were treated with 0.25 mgm. (two with 2.5 mgm.) twice daily for 3½ days. Table IV shows that strain 6 was passaged, during 17 weeks, through 20 serial infections, nine of which were treated, usually with 5.0 or 2.5 mgm. twice daily for 3½ days.

TABLE V  
Comparison between response to paludrine of the parent strain and of paludrine-resistant strains 5 and 6 respectively

Strain	Results at							M.E.D. (mgm.)	Re- sistant factor* (approx.)
	8 mgm.	4 mgm.	2 mgm.	1 mgm.	0.5 mgm.	0.25 mgm.	0.1 mgm.		
Parent Pal.-res. 5		18/246	101/246	122/246	340/336	1/321 406/336	44/321 366/336	0.1-0.25 4	20
Parent Pal.-res. 6	Lethal	84/352	198/352	365/332	346/332	4/252 369/332	49/252	0.1-0.25 4-8	20-40

$$\text{*Resistant factor} = \frac{\text{M.E.D. for resistant strain}}{\text{M.E.D. for parent strain}}$$

The results are given as in Table II.

The response of the strains to paludrine was then tested by Davey's technique, and, as shown by Table V, strain 5 proved to be about 20 times, and strain 6 about 20-40 times, as resistant as normal. In fact, in the case of strain 6 the resistance was such that the maximum doses tolerated by the chicks only barely influenced the infections. The very high degree of resistance shown in these tests may well have been attained at a very much earlier stage—perhaps when resistance first became apparent as at least a tenfold increase after 8-11 treatments in strain 5, and after five treatments in strain 6, as described above—but the exact degree of resistance was unfortunately not measured at those earlier stages.

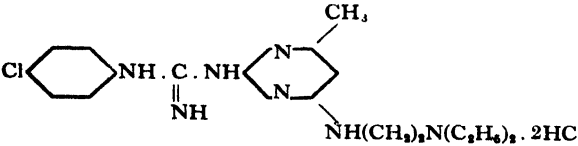
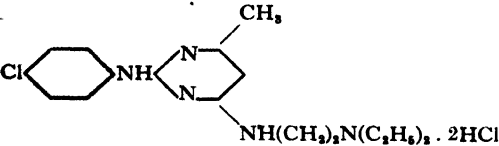
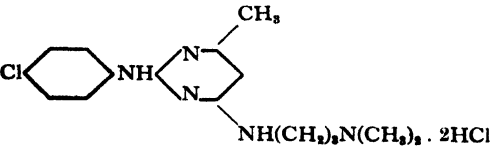
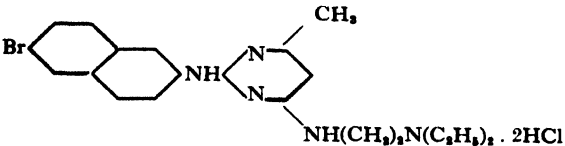
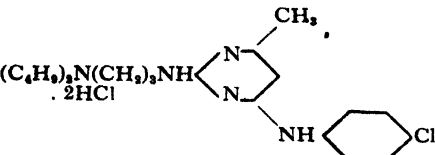
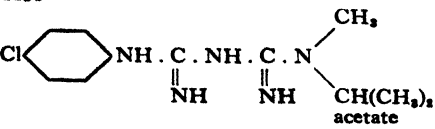
#### TESTS OF THE PALUDRINE-RESISTANT STRAIN FOR RESISTANCE TO OTHER ANTIMALARIALS

The results of tests for cross-resistance between the paludrine-resistant strain (strain 6) and a number of other antimalarials are set forth in Table VI. The table shows that the production of paludrine-resistance does not confer resistance to the entirely unrelated older compounds, mepacrine, quinine or sulphadiazine, or to various pyrimidine precursors of paludrine, but that it does confer complete resistance to 4430, the N<sub>6</sub>-methyl analogue of paludrine (Curd, Davey and Rose, 1945b).

At the conclusion of these investigations no morphological differences were observed, in untreated infections, between the parent and any of the previously treated strains 1-6.

TABLE VI

Tests for cross-resistance between paludrine-resistant strain 6 and various other antimalarials

Drug tested	Strain tested	Results at			M.E.D.
		4 mgm.	2 mgm.	1 mgm.	
Mepacrine methanesulphonate ... ..	Parent Pal.-res.	0/456	15/453 1/456	191/453 173/456	2 2
Quinine hydrochloride ... ..	Parent Pal.-res.	0/318	5/471 0/318	46/471 110/318	2 2
Sulphadiazine ... ..	Parent Pal.-res.	3/415	25/417 68/415	235/417 50/415	2-4 2-4
3349					
 <chem>ClC1CCCCC1NCC(=N)N(C)CCN(CC)CC</chem> . 2HCl	Parent Pal.-res.	3/420	61/429 64/420	366/429 371/420	4 4
2666					
 <chem>ClC1CCCCC1NCCN(C)CCN(CC)CC</chem> . 2HCl	Parent Pal.-res.	18/433 *40/383	323/433 312/383		4 2-4
3711					
 <chem>ClC1CCCCC1NCCN(C)CCN(CC)CC</chem> . 2HCl	Parent Pal.-res.	6/397	82/354 80/397	204/354 379/397	4 4
3502					
 <chem>BrC1CCC2CCCCC2N1CCN(C)CCN(CC)CC</chem> . 2HCl	Parent Pal.-res.	2/366	46/386 51/366	283/386 367/366	4 4
4316					
 <chem>CCN(CC)CCNCC(=N)N(C)CCN1CCCCC1Cl</chem> . 2HCl	Parent Pal.-res.	3/448	73/454 90/448	421/454 309/448	4 4
4430					
 <chem>ClC1CCCCC1NCC(=N)NC(=N)N(C)CCN(CC)CC</chem> acetate	Parent Pal.-res.	238/443	418/443	3/427† 377/443	1 >4

\* Result at 8 mgm. : 2/383. † Result at 0.5 mgm. : 228/427.  
The results are given as in Table II.

## DISCUSSION

## RELATIONSHIP TO MECHANISMS OF DRUG ACTION

The relatively rapid development of a high degree of acquired resistance to paludrine is especially remarkable in view of the failure to produce resistance to other antimalarials by their administration in intensive dosage over very long periods. Although insusceptibility of the exo-erythrocytic stages of the parasite to the action of a drug (e.g., mepacrine or quinine) may well be a factor in protecting a strain from the development of resistance to that drug, it is evident that susceptibility of these stages (e.g., to paludrine) is not, of itself, sufficient to confer a potentiality towards the ready development of resistance. If this were the case, then some degree of resistance should have been produced by our prolonged administration of sulphadiazine, which does exercise substantial activity against exo-erythrocytic forms of *P. gallinaceum*. There seems to be no clear pointer as to why one particular drug and not another is liable to give rise to resistance.

It is not surprising that acquired paludrine-resistance does not automatically involve an acquired resistance to the older compounds, mepacrine, quinine or sulphadiazine. Although little is yet known of the precise modes of action of any of these antimalarials, such evidence as is available does support the safe assumption that the considerable structural differences between paludrine and the other compounds reflect radically different modes of action. It would therefore not be particularly expected that resistance to paludrine should confer resistance also to the other types. Nevertheless, wide differences in chemical constitution do not necessarily rule out the possibility that resistance to one type of compound should comprehend resistance also to another, as shown by the case of acquired resistance in trypanosomes to aromatic arsenicals, such as atoxyl, involving automatically a similar resistance to entirely unrelated non-arsenical acridine compounds (Ehrlich, 1909; Yorke, Murgatroyd and Hawking, 1932). The point could therefore be definitely settled only by actually putting it to the test.

Again, it is not surprising that paludrine-resistance should involve resistance also to 4430, the N<sub>6</sub>-methyl analogue of paludrine. These two compounds are so alike chemically that they must surely exercise their lethal action on the parasite by closely similar mechanisms, so that a resistance acquired to the one might be expected to operate also against the other.

However, it is perhaps surprising that acquired resistance to paludrine does not confer similar resistance to its pyrimidine precursors such as 3349, 2666, etc. There is probably some relationship between this and our finding that, whilst resistance may be produced fairly rapidly by the use of paludrine, no resistance is produced by very prolonged and intensive dosage with 3349. The widely divergent behaviour in regard to drug-resistance between the paludrine and 4430 type of compound, on the one hand, and the precursor-type, 3349, 2666, etc., on the other, may be related to either or both of two obvious features which differentiate these two types of compound chemically. These are the presence in the latter type, but not in the former, of (a) the pyrimidine ring, and (b) a dialkylaminoalkylamino side-chain. Which (if either) of these two features is concerned with the differences of behaviour, in regard to drug-resistance, it is not possible to say. The fact that the dialkylaminoalkylamino side-chain is an essential feature of mepacrine and pamaquin, and that resistance to the former has not, and to the latter has only with difficulty (Bishop and Birkett, 1947), been obtained in *P. gallinaceum* infections,

suggests that this side-chain, rather than the mere presence of the pyrimidine ring, may be the determining feature, though the manner in which it might exercise its influence remains obscure. It may well be related, in some way, to differences in mechanism of adsorption or fixation of the drug on the parasite, rather than to differences in the mechanisms by which the ultimate lethal stroke is delivered by drug on parasite.

Curd, Davey and Rose (1945*b*) stressed the hypothesis that the possibility of certain types of prototropy is an essential prerequisite for antimalarial activity in the compounds which led from their pyrimidine prototypes towards paludrine. Since this feature is, then, common to all the compounds which exhibited activity, whether pyrimidines of the type of 3349 or biguanides of the type of paludrine, it seems reasonable to suppose that the ultimate mechanism of parasitocidal action must be shared by all these compounds. Inherently likely though this conception may be, it is hardly compatible with the tentative theory of Curd and Rose (1946) that paludrine may act by interfering with the parasite's porphyrin metabolism through the *in vivo* formation of a chelated paludrine complex simulating certain natural porphyrins. It is not likely that any of the active pyrimidine compounds can form complexes which simulate porphyrins as closely as do the chelated paludrine molecules. And certainly the mere ability to form such porphyrin-simulating complexes is insufficient to confer antimalarial properties, as shown by certain benzimidazoles related to paludrine, which are capable of forming such complexes, but which do not exercise any antimalarial activity (Acheson, King and Spensley, 1947). Incidentally, we have sought to test, indirectly, the validity of the Curd-Rose porphyrin-interference theory of paludrine activity, by administering large doses of BAL (British anti-lewisite) (Peters, Stocken and Thompson, 1945) to chicks undergoing paludrine treatment for *P. gallinaceum* infections. The idea was that since BAL has an outstanding affinity for heavy metals (see also McCance and Widdowson, 1946) it might compete with paludrine for available metal. In this way, it would perhaps interfere with formation of the porphyrin-simulating chelated paludrine complex, and might accordingly interfere with the therapeutic activity of paludrine. However, no such therapeutic interference was observed. Needless to say, negative experiments of this kind are insufficient to invalidate the theory under consideration. They do no more than merely to fail in supporting it, but are, perhaps, none the less worthy of placing on record, in passing.

On the whole, we incline to the view, stated above, that paludrine and the active pyrimidine compounds are likely to share a common essential mechanism of parasitocidal action, though there may be very considerable differences in mechanisms of fixation of the drug on the parasite, and also, no doubt, in other details of the physico-chemical steps which lead to the ultimate lethal effect. These differences would doubtless result in differences between one type of drug and another (indeed even between different drugs of the same type) in the detailed pattern of drug action, reflecting themselves in specificity of drug action, whilst the ultimate lethal blow is delivered in the same manner by the one type as by the other.

Such a concept is paralleled by our recent work with *Trypanosoma rhodesiense* and  $\gamma$ -(*p*-arsenosophenyl)butyric acid, which differs radically from other arsenoxides which are not carboxyl-substituted, in that it is fully active against trypanosomes which have

\* For example, activity of 2666 and inactivity of 3349 against *P. lophurae* (Curd, Davey and Rose, 1945*a*); activity of paludrine and inactivity of 4430 against *P. cathemerium* (Curd, Davey and Rose, 1945*b*).

been made resistant to the other arsenoxides (Eagle, 1945 ; Williamson and Lourie, 1946). We produced evidence that this property does not reflect an essential difference between *p*-arsenosphenylbutyric acid and the other types of arsenoxide in the ultimate means by which they destroy the trypanosome, which in both cases appears to be by an inactivation of sulphhydryl groups of the trypanosome cell. Further, our experiments produced circumstantial evidence for the belief that the essential difference between these two types of compound in their attack upon the trypanosome—the difference which no doubt determines their divergent behaviour in regard to drug-resistance—is that they have different mechanisms of fixation on, or entry into, the trypanosome cell. Earlier work by King and Strangeways (1942) and King (1943) gave the strongest support for this view.

We suggest, then, that a similar basis is likely to underlie the widely divergent behaviour, in respect of drug-resistance, between the paludrine and the pyrimidine precursor types of compound. The suggestion is that the ultimate lethal stroke (the nature of which in the case of these antimalarials is as yet unknown) is the same for both types, whilst considerable differences may exist in the mechanisms by which each becomes attached to, or enters, the parasite cell. It is, therefore, these presumed differences in fixation of drug on parasite, rather than any basic differences in the mechanism of lethal action, which may be essentially related to the differences observed between the two types of compound in the matter of acquired drug-resistance.

#### RELATIONSHIP TO HUMAN MALARIA

The potential significance of this work, in regard to the use of paludrine in human malaria, is of obvious and of very considerable importance. It is not possible, and it would be most rash, at the present stage to attempt any prediction as to whether or how readily the widespread use of paludrine is likely to result in the production and propagation of resistant strains of human malaria in the field. The work undoubtedly shows that such an eventuality is, at the least, a theoretical possibility, whilst similar possibilities in regard to mepacrine or quinine may be dismissed as highly unlikely. A necessary element in reinforcing this conclusion is the fact, as will be elaborated in a later contribution in this series, that paludrine-resistance is retained after passage through mosquitoes—a fact which, in any case, is of far-reaching biological interest.

The case of arsenic-resistance in pathogenic trypanosomes illustrates how such a theoretical possibility has, in fact, been translated into actual effect. Van Hoof (1947) has recently described how, in the Belgian Congo, where mass treatment with arsenicals for sleeping sickness has long been in vogue, the incidence of tryparsamide-resistant cases increased, in the course of a few years, from about 7 per cent. to 50 per cent. or more in certain districts. Again, in the case of pathogenic bacteria which are normally susceptible to the sulphonamides—at any rate in the case of gonococci—there is increasing evidence that the well-known potentiality of these organisms to acquire sulphonamide-resistance is now being reflected, in clinical practice, by a greater incidence than formerly of infections refractory to treatment with these compounds.

If there is indeed a danger that the widespread use of paludrine may result in the establishment of paludrine-resistant strains of human malaria, the likelihood of such an outcome is probably greater in the case of *P. vivax* than in *P. falciparum*, since it appears that benign tertian malaria is much less readily sterilized by paludrine than is malignant tertian malaria (Macgraith *et al.*, 1946 ; Fairley *et al.*, 1946). If the hypo-

thetical danger of the production of paludrine-resistant human malaria will eventually materialize, it is an especially fortunate circumstance that strains which have developed a resistance to paludrine should, as our observations indicate, retain unimpaired their sensitivity to mepacrine, quinine and certain other antimalarials.

It must be emphasized, however, that it is very easy to over-estimate the weight of our investigations in their bearing on the question of whether paludrine-resistant strains of human malaria are likely to be established by the normal use of paludrine in treatment and prophylaxis in man. Our development of paludrine-resistance has been brought about under highly artificial conditions, in syringe-passaged bird malaria, where various factors, such as the timing of treatments, were deliberately calculated to favour the rapid development of resistance. We must stress, therefore, that, as regards human malaria, our experiments have merely established that the eventual development and propagation of paludrine-resistant strains is a more likely theoretical possibility than is the similar production of mepacrine- or quinine-resistant strains; but much further experimental work would be necessary before it could be suggested that the danger looms large.

### SUMMARY

1. *Plasmodium gallinaceum*, in four lines of serially infected chicks, was subjected for nearly 2½ years to intensive treatment with, respectively, mepacrine, quinine, sulphadiazine and 3349 (a pyrimidine precursor of paludrine). No resistance was acquired to any of the four compounds used.

2. Similar treatment with paludrine, either in the minimum dosages effective for normal infections or in the maximum tolerated by the host, gave rise in about three or four months to a high degree of paludrine-resistance, transmissible through *Aedes aegypti*. In the series treated by high doses the resistance appeared suddenly, after the strain had been passaged through four treated infections and in circumstances which suggested the selection of a mutant differing widely from the normal, rather than the gradual training of parasites to slowly increasing degrees of resistance.

3. A strain made resistant to paludrine is also resistant to the analogous biguanide 4430, but not to their pyrimidine prototypes, such as 3349, 2666, etc. Nor is it resistant to mepacrine, quinine or sulphadiazine.

4. The widely divergent behaviour of the biguanides and of their pyrimidine precursors, in the matter of acquired resistance, does not necessarily point to any essential difference in their modes of drug action. These may be basically similar, whilst differing in detail, perhaps through differences in adsorption or fixation on the parasite cell. Reasons are given for suggesting that it might be differences of this nature which underlie the differences of behaviour in regard to acquired resistance.

5. In its bearing on human malaria, this work does no more than to establish that the development of paludrine-resistant strains is a more likely theoretical possibility than the similar production of mepacrine- or quinine-resistant strains. It provides no basis for suggesting that the eventuality is a probable one.

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# EXPERIMENTAL STUDIES OF AN OLD STRAIN OF *TRYPANOSOMA GAMBIENSE*

## I.—THE ENHANCEMENT OF ITS VIRULENCE AND THE RELATIONSHIP OF THIS PHENOMENON TO THE SPECIES OF POLYMORPHIC TRYPANOSOMES OF AFRICA

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### INTRODUCTION

Ever since Bruce's discovery in 1896 of a haemoflagellate, later named *Trypanosoma brucei* Plimmer and Bradford, 1899, as the causal agent of the fatal 'nagana' disease of cattle and horses in Zululand, and the subsequent incrimination of *T. gambiense* Dutton, 1902, in the aetiology of West African sleeping sickness, and of *T. rhodesiense* Stephens and Fantham, 1910, in the production of the more acute form of trypanosomiasis, the genetic relationship between the three so-called 'polymorphic' species of trypanosomes has been a matter of controversy.

Extensive studies in the laboratories of Europe, followed by comprehensive enquiries by Commissions of the Royal Society and the League of Nations, as well as much arduous and frequently heroic field-work (cf. Fairbairn and Burtt, 1946) on the infectivity of different strains of trypanosomes isolated from man and other animals, including the tsetse-fly vectors of the parasite, have settled a number of formerly disputed points. It is now generally agreed that no constant differences exist on the morphological level between these three presumptive species (Hoare, 1936; van Hoof *et al.*, 1944).

On purely morphological criteria, therefore, only a single species could be recognized. For reasons of priority this would be *T. brucei*. On cogent clinical, epidemiological and economic grounds, however, the 'polymorphic' trypanosomiasis of Africa have been referred to distinct, though not entirely discrete, categories. Thus, *T. gambiense* infection is usually a chronic disease, sometimes symptomless and seemingly self-curative (Barlovatz, 1934), and nearly always presenting a relatively low-grade parasitaemia. It occurs in relatively densely populated regions having a correspondingly small wild (mammalian) fauna. The disease, especially in its early stages, is amenable to treatment with such pentavalent arsenical drugs as tryparsamide, etc.

These circumstances are in strong contrast to those prevailing in the *T. rhodesiense* type of infection. This is a more fulminant disease, characterized by a high parasitaemia and refractory to pentavalent arsenicals, but, like *T. gambiense*, susceptible in the early stages of the infection to the non-metallic drugs such as antrypol (syn. Bayer 205).

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Untreated, the disease runs a rapidly fatal course. It is more likely to be encountered on the edge of sparsely populated bush-country with a large wild fauna, thus giving support to the old idea that big game serve as reservoirs of the virus.

Several of the clinical and parasitological characteristics of the human disease are stable enough to be duplicated in appreciable measure when the trypanosomes involved are subinoculated into rats and mice.

Concerning the species relationship, Wenyon (1926, p. 549) surmised that *T. gambiense* 'originated from *T. brucei* of animals, it may be centuries ago, but that having passed from man to man through many passages, has become modified morphologically (disappearance of posterior nuclear forms) and as regards its virulence for laboratory animals. The human strain of *T. brucei*, on the other hand, represents the animal strain which has only recently infected man, and, having been subjected to few passages, still maintains its morphological characters and virulence. *T. gambiense* is sufficiently distinct to be regarded as a species, but *T. rhodesiense* is merely a strain of *T. brucei* in man.'

Brumpt (1936, p. 310) deals with the species relationship of *T. gambiense* as follows (my translation): 'Certain authors consider this trypanosome to be a strain of *T. rhodesiense* which has become less virulent in the course of transmission by *Glossina palpalis*. Others go further than this, claiming that the two trypanosomes parasitic in man are identifiable with *T. brucei*. This latter species adapts itself under our very eyes to enfeebled subjects, becoming what we would call *T. rhodesiense*. This brilliant and plausible concept, despite its philosophical interest and the rôle it may play as a working hypothesis for research, has no practical interest because it is invalidated by all experimental and epidemiological facts.'

Enough has been learned from laboratory investigations to show that neither the infectivity nor the virulence of trypanosomes is an immutable character stabilized beyond modification by events in their past history. For example, Wenyon (1926, p. 523) describes one of the cattle trypanosomes, *T. congolense*, as being 'Virulent for all laboratory animals. After passage through goat loses virulence for laboratory animals, and then resembles the natural strain, *T. nanum*, which is not inoculable to laboratory animals.' In another place (p. 513) he draws attention to the fact that 'There is a variation in the animals inoculable with any one trypanosome, and, furthermore, after successive passages the virulence may become much increased.'

The older literature describing the behaviour of various strains of *T. gambiense* and *T. brucei* records changes in virulence undergone by these species when passed through various animals over a prolonged period. Even though several hypotheses have been presented to account for such changes, little has been learned definitely of the mechanism underlying the phenomenon. It is true that a certain degree of enhancement or debasement of virulence has occurred in some laboratories in the course of maintaining strains of *T. brucei* and *T. rhodesiense* that *ab initio* were already fairly pathogenic (cf. Yorke *et al.*, 1933; Murgatroyd and Yorke, 1937), but no procedure appears to have been devised which can be depended upon regularly to increase the virulence of a species, such as *T. gambiense*, which normally is only slightly pathogenic to laboratory animals (cf. Corson, 1936, 1937; Vanderplank, 1941; Burt, 1946).

In the following pages we present an account of the behaviour in the laboratory of a typical strain of *T. gambiense* and of a procedure which was elaborated to render the organism highly pathogenic for certain laboratory rodents.

### HISTORY OF A STRAIN OF *T. GAMBIENSE*

The 'Nzale' strain was brought to the United States in 1944 by Dr. David Weinman, at that time of Harvard University, who secured it from Dr. C. Henrard, of the Institut Princesse Astrid in Leopoldville, Belgian Congo. According to Weinman, the strain was originally isolated from a wild *Glossina palpalis*, caught in the vicinity of Leopoldville and induced to bite a guinea-pig. Subsequently, it is stated to have been passed through other guinea-pigs, a monkey and a man (*sic*). In Dr. Weinman's laboratory the strain again passed through a number of guinea-pigs and an occasional rat which, we were informed, 'developed a rather light infection which was not at all or only slightly pathogenic.' The strain was distributed in the United States under the aegis of the American Academy of Tropical Medicine, and, through the courtesy of Dr. A. J. Walker, of the Department of Tropical Medicine at Tulane University, came to our laboratory early in 1946 in a guinea-pig. Although no details of the behaviour of the strain in man or monkey are available to the writer, the strain appears to have been regarded as a typical *T. gambiense*.

The first guinea-pig examined by us 10 days after infection usually showed 2-3 trypanosomes per cover-glass preparation of the blood. The animal died in 40 days, but before death a substantial quantity of blood taken from the heart was injected into another guinea-pig and into a series of rats, in which it was intended to test a series of compounds which had previously shown good trypanocidal action against *T. equiperdum*, *T. hippicum* and *T. brucei*.

### COURSE OF INFECTION WITH *T. GAMBIENSE* ('NZALE' STRAIN) IN VARIOUS RODENTS

Intraperitoneal injection of citrated blood containing an estimated million trypanosomes was used as a routine in our laboratory to produce what we refer to as our standard infection. Unless otherwise stated, all infections referred to in this paper were made in this manner. Thin blood films were usually examined on five or six days a week, and the intensity of the parasitaemia was recorded by the numbers of trypanosomes found at a microscope magnification of 430 (ocular  $\times 10$ ; objective 4 mm.). The following account briefly summarizes our findings.

*Guinea-pigs.* Twenty-three guinea-pigs were infected in series. Their average longevity was 78 days, with extremes of less than 25 days in two instances (in which death may have been due to causes other than trypanosomiasis) and more than 160 days in four instances. In general, a few trypanosomes (+ according to our scheme of scoring) were demonstrable in the blood of the majority of the guinea-pigs within a week of inoculation. The parasitaemia is recorded as continuing scanty for several weeks; at times no parasites at all were found in blood smears. Our records for many guinea-pigs show a rising parasitaemia after about a month (++ to +++). In one animal (scored ++++) a haemocytometer count\* showed 400,000 trypanosomes per c.mm. of blood. High parasitaemia in guinea-pigs is usually of brief duration and is followed by a variable period of negative blood examinations. One, two or even three waves of increasing parasitaemia may be seen on the charts of some guinea-pigs before they died. On the

\* I am indebted to Mr. Max MacCowan for making many of these haemocytometer counts.

other hand, some animals died without more than a few trypanosomes having been found on rare occasions, in spite of frequent blood examinations.

As a rule, infected guinea-pigs presented no symptoms of disease. They fed normally, increased in weight, and even gave birth to uninfected young (two instances). The infection of guinea-pigs with this strain of *T. gambiense*, despite the inevitably fatal outcome, must in our opinion be described as chronic and 'mild.'

*Rabbits.* Irrespective of the size of the recipient rabbit, or of the number of trypanosomes (between 1,000,000 and 100,000,000) used to induce infection, or of the specific source or donor of these organisms, the course of infection in this host was very benign. Trypanosomes were rarely seen in the peripheral blood, and then only in small numbers. In some rabbits we were never able to find trypanosomes; nevertheless, subinoculation of heart's blood from such rabbits into appropriate test rats (see below) proved the persistence of infection. Infected rabbits were kept under observation for as long as five months without showing signs of disease; after that time they were either discarded because of limited cage-accommodation or reinfected for immunity studies. One rabbit infected with the 'Nzale' strain died after 18 days with pneumonic consolidation of the lungs, evidently not due to trypanosomiasis.

*Hamsters (Cricetus aureus).* Five laboratory-bred hamsters were infected with trypanosomes of the 'Nzale' strain derived either from guinea-pigs or from rats. All became infected, but in none did trypanosomes ever become numerous enough in the blood to be scored more than +. The infection was completely asymptomatic, and all five hamsters were discarded in an apparently healthy state after three months' observation.

*Mice.* The course of infection was closely followed in 27 'Swiss' mice in four lots, each infected with trypanosomes of rat or guinea-pig origin. In one lot of four mice examinations over a period of three months failed to reveal a single trypanosome. In another lot of 10 mice parasites were found sparingly within the first week of infection, but smears made thereafter were negative. Nevertheless one of these superficially trypanosome-free mice was finally shown to harbour infection by injecting an emulsion of its brain into rats. Most of the mice were discarded after 100 days, although within that period a number had died, presumably from causes other than trypanosomiasis.

*Rats.* From the outset of these studies we used adolescent rats weighing between 80 and 120 gm. (35 to about 50 days old) belonging to the Wistar and Evans strains; infection was started initially from our original 'Walker' guinea-pig and subsequently from other rats. Irrespective of the size of the infecting inoculum (i.e., between the limits of 10,000 and about 10,000,000 trypanosomes), or of whether these were derived from early or late phases of the donor's infection, rats rarely, if ever, showed signs of trypanosomiasis. In 50 rats, whose blood was examined at frequent intervals over the entire span of infection, trypanosomes were sparse or even absent for the first month or two following inoculation. Thereafter fairly heavy (++) to very heavy (++++) parasitaemias were observed in about half the group, whereas the remainder never showed an intensity of parasitaemia which could be scored higher than +. The intense parasitaemias which merited ++++ scores were found to involve numbers of trypanosomes ranging from 1,410,000 to 3,450,000 per c.mm. of blood. Sometimes these high-grade parasitaemias were accompanied by pronounced anaemia (erythrocyte counts of 3,500,000 c.mm., the normal red blood-corpuscle count in the albino rat being in the range of 9-10 million c.mm.) and a gradual loss in weight prior to the death of the host;

but usually one would not suspect from the animal's appearance or behaviour that its blood was teeming with parasites. In such instances the trypanosomes often disappeared from the blood over night ; weeks later a second swarming of the blood with trypanosomes would be followed by the rat's death. However, in some rats we have seen as many as four 'number-crises' before they succumbed to infection. On the other hand, a fair number of rats survived for more than 14 months without showing any signs of infection other than an occasional trypanosome. Infected females have bred and raised their litters, which, incidentally, were in no instance found to have become infected *in utero*. Less than 10 rats died within the first 90 days of infection (minimum 35 days). For 31 rats whose death we ascribed to trypanosomiasis the mean survival time was 166 days ; the average survival time for 60 'Nzale' rats was over 196 days, including a group of four that were still living more than 15 months after inoculation and in whose blood trypanosomes could on occasion be detected either by the microscope or by the subinoculation method.

#### ATTEMPTS TO ENHANCE THE VIRULENCE OF THE 'NZALE' STRAIN

A strain of *T. gambiense* as benign to laboratory animals as that described above is of very limited value for chemotherapy tests because of the difficulty of evaluating results. We have already mentioned that changes in virulence affecting trypanosomes in the laboratory have, in the majority of instances, occurred under circumstances which cannot be properly described as controlled experimentation. Procedures recorded in the literature for enhancing the virulence of strains of African trypanosomes include the following:

1. Mechanical inoculation (i.e., by syringe rather than by infection with cyclical trypanosomes injected by tsetse-fly) of such large numbers of trypanosomes that the natural resistance of the recipient host will be overwhelmed.\*

2. Rapid subinoculation of trypanosomes taken from an early multiplicative phase of the infection in the donor host into (a) hosts of the same species (e.g., mice to mice), or (b) hosts of different species (e.g., mice to guinea-pigs, etc.).

3. Reduction of the power of the host to produce protective antibodies by such procedures as splenectomy or blockade of the reticulo-endothelial system with carbon or colloidal metals.

These and various other procedures were exhaustively explored in our laboratory without producing an evident increase in virulence. Finally, when we were about to abandon all hope of being able to employ this 'Nzale' strain of trypanosome for purposes of drug assay, a new vantage-point appeared.

Studies on the relationship between host-parasite specificity and various manifestations of immunity to infection with metazoan parasites led the writer several years ago to describe the phenomenon of 'age-resistance' in terms of an evolutionary phylogenetic association between host and parasite and to formulate certain generalizations (Sandground, 1928, 1929). It was then postulated that 'age-resistance' to infection denotes a relatively recent and 'abnormal' consortium between the host species and the particular species of parasite involved. If this were true, from the converse one would deduce that the degree of resistance would decrease with a diminution in the physiological age of the abnormal host individual.

\* In our opinion the basis for this postulate is essentially erroneous, in that, while the host's resistance may be subdued by an overwhelming infection, the virulence of the parasite need not necessarily be increased thereby.

The deduction is found to be entirely valid for many parasites in addition to the metazoan forms discussed in our original paper. For instance, it applies in the case of the South American *T. cruzi*. The usual or, at least, common vertebrate hosts of this species in nature are certain archaic marsupial and edentate mammals, such as the armadillo. In these 'natural' hosts the parasites are described as occurring in the blood-stream in large numbers and over prolonged periods. Here they are presumably non-pathogenic. In the human host, it will be recalled, Chagas first described the parasite in the blood of infants, at which age the infection runs an acute turbulent course. This is to be contrasted with the chronic cryptic type of infection (low-grade parasitaemia) which occurs in older persons.

The course of *T. cruzi* infection in laboratory animals varies with the species. Mice, as a rule, are more susceptible than rats, which are described as completely refractory to most strains of the parasite. Regendanz (1930), working with an old laboratory strain of *T. cruzi*, virulent for mice, guinea-pigs and dogs, but essentially non-infectious for adult white rats, found that the very young rats were susceptible to infection and developed unusually high parasitaemia when infected before the 17th day of age. (It may be worthy of note that the strain used by Regendanz does not appear to have been modified to the point when it was infectious for rats more than six weeks old.)

Keeping these considerations in mind, we embarked upon a systematic study of the behaviour of the 'Nzale' strain of *T. gambiense* in rats younger than those which we had previously been accustomed to use for routine experimentation.

In the first experiment 10 nursling rats, 17 days old and weighing 30 gm. on the average, were infected with a heavy suspension of trypanosomes taken from an adult rat at a time late in the infection, when its blood was heavily laden with parasites. When examined three days later, five of the nursling rats registered moderate to heavy (+ + to + + +) parasitaemias. This was a novel experience with this strain of trypanosomes. Subsequent daily blood examinations showed only slight fluctuations in the numbers of trypanosomes. One of the baby rats died 18 days after inoculation and the remainder at varying intervals up to 71 days. The average survival time for the 10 nursling rats was 32 days.

A second litter of five 12-day-old rats was infected from a nursling of the first series. Two died with heavy parasitaemias on the 11th day after infection, but the average longevity of this series was prolonged to 43 days by the survival of one rat for 94 days.

The third passage comprised only three rats, 11 days old and infected from the second series. These three rats died on the 8th and 9th days following infection; their parasitaemias developed without fluctuation in numbers and in the manner characteristic of a highly virulent strain of trypanosomes. From this point on there was a gradual increase in the virulence of the strain until the 7th serial passage, when the nursling rats were regularly dying in as short a time as three days.\*

After the 6th passage, a virulence-test was undertaken. Three 40-day-old rats were infected with 1,000,000 trypanosomes derived from a nursling rat. These rats developed heavy parasitaemias and were all dead on the 4th day of the experiment. On repeating

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\* Control nursling rats for all these serial passages, kept in the same cage with their infected siblings and nursing mother, were usually successfully weaned. Occasionally a mother-rat turned cannibal and devoured her young, in which event, of course, a new start had to be made.

the test with rats weighing up to 350 gm. (up to two years of age) a similar result was obtained.

The virulence-enhancement experiment described above was by this time regarded as successful. The strain was subsequently maintained by subinoculations into adult rats or adult mice. It was referred to as the 'V' (for virulent) strain, to distinguish it from the avirulent 'Nzale' strain from which it stemmed. Throughout this period the latter strain was also conserved in rats and guinea-pigs, in which it continued to manifest its original non-pathogenic character.

#### COMPARISON OF THE BEHAVIOUR OF THE 'NZALE' STRAIN AND THE DERIVED 'V' STRAIN OF *T. GAMBIENSE* IN VARIOUS LABORATORY ANIMALS

In order to demonstrate succinctly the change of virulence wrought in the 'Nzale' strain a series of parallel infections were started in various host species.

*Mice.* A group of 10 adult mice infected with 'V' strain trypanosomes began showing a few organisms in the blood within 24 hours. In each mouse the parasitaemia increased progressively, until the blood, on the 3rd day of infection, was swarming with flagellates, estimated by haemocytometer count to number up to 2,000,000 per c.mm. of blood. Some mice were dead on the 4th day; all were dead by the 5th day; and among several hundreds of mice used for passage of this strain not a single animal survived more than eight days.

Twenty 'Nzale'-strain infected mice, in groups of 10, served as controls. Among these only four deaths occurred in 30 days before the animals were discarded. A very low-grade parasitaemia was detected during the observation-period in eight of these mice; in the remainder trypanosomes were never found.

*Rats.* In each of a group of 10 adolescent rats (100 gm. weight) infected with the standard dose of 'V' strain trypanosomes, parasites appeared in the blood within 24 hours. By the 3rd day the parasitaemia was scored ++ or ++++. The 5th day of the experiment marked the death of the first rat in the group; all were dead by the 7th day. Our protocols show that, among several hundreds of adult rats infected either as 'seed' animals or as controls for chemotherapy trials, not one had lived for more than nine days; some are recorded as dying in as short a time as three days.

In contrast to this, evidence of blood invasion could be found in only five of 10 'Nzale'-strain infected rats by the 8th day; in two more trypanosomes were detected on the 12th day, and it was not until the 15th day that they were seen in the blood of the 10th member of the group. Only in the agonal stage of infection was a +++ parasitaemia recorded in any of these rats. One rat died on the 25th day of the experiment; the other nine died at irregular intervals up to the 95th day.

*Hamsters.* We have already recorded the benign course of infection with the 'Nzale' strain in hamsters. The contrast in the reaction of this host to 'V' strain trypanosomes is very distinct, but, strangely enough, the distinction is on the pathogenic side, without an accompanying change in the parasitaemia. The general appearance of well-being in 'V'-strain infected hamsters begins to deteriorate within a few days of infection; the fur becomes ruffled; the animal shows evidence of central nervous system involvement in its increased irritability; it loses weight, develops diarrhoea, and ultimately may become blind or paralysed in the hind quarters. The average survival time among 12 'V' infected hamsters was 16.8 days (range 10-30 days).



Five 'Nzale' infected hamsters were discarded in an apparently healthy condition after three months of observation.

It may be interesting to observe that, despite the pathogenicity of 'V' strain trypanosomes for hamsters, parasites were only occasionally found in their blood and always in small numbers.

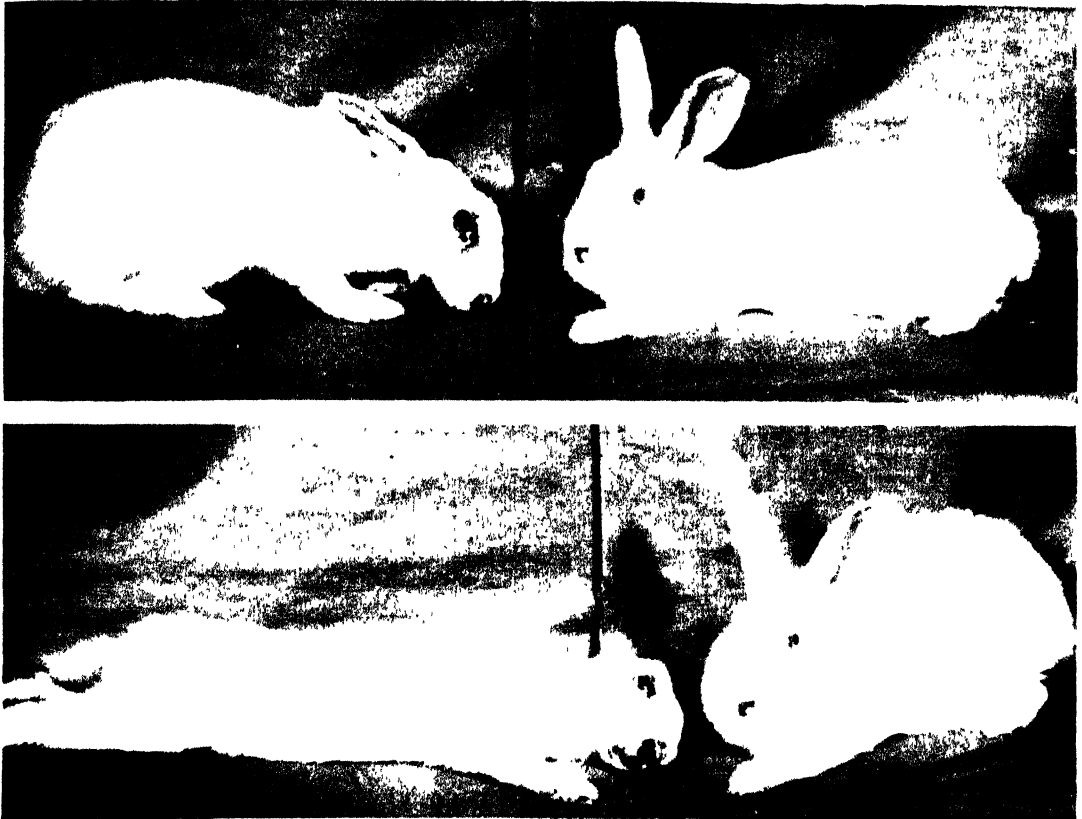
*Rabbits.* As illustrated in Plate XI, 'V' strain trypanosomes produce a most striking pathognomonic picture in rabbits, in contrast to the seemingly avirulent effect of the 'Nzale' strain organism. Within a week of inoculating rabbits with the standard dose of 'V' organisms, there is a noticeable elevation in temperature. In white rabbits, particularly, a swelling of the periorbital tissues and a peculiar thickening of the ear margins has been observed. One rabbit developed a cloudiness of the cornea, and appeared to be blind before it died. The animals lie prostrate and apathetic in their cages for several days before death. Lymphadenopathy and a gross atrophy of the cardiac musculature were the most obvious and consistent changes found at post-mortem examination. Of four rabbits infected with 'V' strain trypanosomes, none lived beyond 60 days; two were sacrificed, respectively on the 28th and 44th days after infection, in a moribund state for a histopathological study. Blood examinations at frequent intervals during the course of the disease only occasionally revealed trypanosomes. In respect to the parasitaemia, therefore, 'V' strain infections are in no way different from 'Nzale' strain infections in rabbits.

*Guinea-pigs.* The course of 'V' strain infection has been found more variable in guinea-pigs than in any of the hosts described above; at the same time the contrast between the effects of 'V' and 'Nzale' strain infections is also less marked. As with the 'Nzale' strain, guinea-pigs infected with 'V' strain trypanosomes seldom show a parasitaemia of more than 400,000 flagellates per c.mm. of blood (+ - on our scoring system), and that only in rare cases. In many guinea-pigs parasites appear in the blood in waves, which are short in duration and separated by long intervals of cryptic infection. This host seldom shows outward physical signs of disease.

It is difficult to make a general statement regarding the grade of virulence of the 'V' strain for this host. The extremes of variation are illustrated by the fate of three guinea-pigs infected with 1,000,000 'V' trypanosomes. One died in 23 days, the second died in 153 days, while the third lived for 186 days. Three guinea-pigs infected as controls in this test were alive and healthy in appearance on the 200th day. An analysis of our consolidated records shows that for 17 'V' strain guinea-pigs the mean survival time was 39.4 ( $\pm$  8.73) days, while the mean survival time of 19 'Nzale' strain guinea-pigs was 46.4 ( $\pm$  13.85) days. Here the numbers of animals are too small and the degree of variation too great for the survival-time figures to carry statistical significance.

#### MORPHOLOGY AND DRUG-SUSCEPTIBILITY OF THE 'V' STRAIN

The transformation of the original 'Nzale' strain of *T. gambiense* described above goes beyond the simple exaltation of its pathogenicity for certain laboratory animals. The change has yielded an organism indistinguishable in its differential susceptibility to particular classes of trypanocidal drugs, as well as in its morphology and general behaviour, from an old strain of *T. brucei* studied in our laboratory for a period of more than five years. Like *T. brucei* in rats, the 'V' strain is sensitive to such non-metallic organic compounds as the symmetrical ureide antrypol (syn. Bayer 205, etc.) and the diamidine



Photographs of two adult rabbits each of which had received intraperitoneal injections of approximately 1,000,000 Trypanosomes of (right) 'Nzale' strain *T. gambiense* derived from rat infection, and (left) the 'V' modification of the 'Nzale' strain, also taken from a rat.

The upper photograph was taken 15 days after inoculation and illustrates the early signs of the disease, viz., oedema of the periorbital and circumnasal tissues, produced by 'V' strain infections.

The lower photograph shows the 'V'-strain infected rabbit in the agonal stages of the disease. There is flaccid paralysis of the limbs and an apparent loss of sight. At necropsy (performed by Dr. C. G. Culbertson, pathologist) this rabbit showed a generalized hypertrophy of the lymph-nodes, together with a marked atrophy of the heart musculature.

The 'Nzale' strain rabbit showed no signs of disease at the end of the third month of the infection.



compound stilbamidine. On the other hand, 'V' strain infections in rats are not affected by the highest tolerated doses of tryparsamide or carbarsone and are only temporarily suppressed by the arsenoxide mapharsen.\* A comparison of the reaction of the 'V' and the 'Nzale' strain infection to the above-mentioned drugs cannot be advantageously made, because, as was stated earlier in this paper, the 'Nzale' strain in rats and mice is too benign and irregular to lend itself to chemotherapeutic assays.

In stained smears the 'Nzale' strain conforms in every way with the morphology of *T. gambiense*. The 'V' strain, especially when the organisms are rapidly dividing at the fastigium of infection, occasionally shows a small proportion of 'stumpy' short trypanosomes without free flagellum—forms that are often described as typical of *T. brucei*. However, it is suspected that these 'stumpy' forms are juvenile trypanosomes, the products of recent binary fissions. We have never encountered 'posterior nuclear forms' such as are often recorded for some strains of *T. brucei* and *T. rhodesiense*. But it is not unusual to see descriptions of both these latter species in which the supposedly characteristic posterior nuclear forms are absent (e.g., Davis, 1931).

#### STABILITY OF THE 'V' STRAIN

Before considering the significance of virulence enhancement here described in terms of human trypanosomiasis problems, it was deemed desirable to learn something of the permanence of the change. Had the virulence changed spontaneously in the routine passaging of the strain from one reservoir host to another (as appears often to have occurred in some European laboratories, using *T. brucei* or *T. rhodesiense*), it would have been natural to ascribe the cause of the change to the routine procedure, and, perhaps, to interpret it as an expression of the inherent tendency of trypanosomes to undergo mutations of this order. In our case, however, enhancement of virulence was being deliberately sought by one means or another, and, after the objective had been achieved, we were still in possession of the parent strain, which apparently is permanently avirulent under the conditions of passage from adult to adult host.

Investigation of the durability of virulence in the 'V' strain was by way of attempting its attenuation. Old large rats from our breeding-colonies, weighing up to 300 gm., were infected in pairs, one rat receiving more than a million trypanosomes by intraperitoneal injection, the other receiving not more than a thousand parasites. While the latent (or cryptic) period was often protracted a day or two longer in the rats infected with the smaller number of trypanosomes, no constant or substantial difference was observed in the time-span of the disease. Thus, the average survival time of 10 rats comprising the initial five serial passages was 5.9 days, as compared with an average of 5.2 days for the 10 rats in the 30th–35th passages.

Incidental to the maintenance of the various strains of trypanosomes in the laboratory, infections were carried in guinea-pigs and rabbits, as secondary safeguards against loss of the strain when shorter-lived 'reservoirs' are used. Even though, as already described, the 'V' strain does not always behave as a virulent organism in guinea-pigs, we have found that its virulence for rats after passage through the guinea-pig remains unimpaired. Transfers from rats or rabbits to mice or hamsters, or various permutations of this order, have likewise failed to cause its attenuation. In its effect upon rats and mice, the strain

\* However, many cures have been effected with *p*-arsenosphenylbutyric acid (Eagle, 1945).

is fully as virulent as the well-known 'ferox' strain of *T. brucei*, so designated by the Ehrlich laboratories where it was first studied. It is indistinguishable also from the Novy strain of *T. brucei*, to be found in laboratories in the United States.

Obviously, unless it can be shown that the results obtained from this virulence-enhancement procedure do not represent a mere coincidence but are substantially reproducible, any consideration of the rôle which the immaturity of the host might play in the 'cause-and-effect' chain of relationships would be inconsequential. For this reason, and also to permit a partial analysis of the phenomenon, the experiment was repeated several times in our laboratory, each attempt being initiated with organisms derived late in the course of infection of a rat with the original 'Nzale' strain. After a shorter or longer series of passages (depending, it would appear, at least in part, on the age of the nurslings) there arose in each instance a strain of which the virulence was greatly exalted above that of the parent strain. In some instances the strain killed adult rats in from 12 to 30 days, resembling in this respect *T. rhodesiense* more than *T. brucei*. Because the results cannot be presented without reference to such ancillary questions as immunity, it is preferable to consider these later experiments in a separate publication.

## DISCUSSION

It will suffice to confine ourselves here to a brief consideration of our present findings in relation to the controversial problem of the taxonomy of Africa's 'polymorphic' trypanosomes.

While attempts at human infection with *T. brucei* derived from various game animals have not been conducted on a scale extensive enough to allow their negative results to be accepted as final and conclusive, some authors, chief among whom is Duke (1936), have interpreted the successful infections which have been induced in numerous species of ruminants and carnivores, both wild and domesticated, with strains of trypanosomes of the *rhodesiense* type originating in man, as support for their contention that game must act as reservoir for the virus responsible for the occasional sporadic cases or the periodic epidemics of sleeping sickness that flare up in Central and East Africa.

In addition, it has been demonstrated that *T. gambiense* of human derivation can infect the domestic pig, in which host it has proved virulent in the experience of van Hoof (see Duke, 1936, p. 293) or may produce an asymptomatic infection such as occurs among wild pigs in West Africa and the Congo.

To the present author it appears that, with the elimination of (a) morphological distinctions (Lavier, 1933), (b) specificity of different tsetse-flies as vectors (Lester, 1933), and (c) serological criteria (Adams, 1933) as means for differentiating between *T. brucei*, *T. rhodesiense* and *T. gambiense*, the only remaining grounds for maintaining these forms as separate specific entities is the dramatic clinical differences presented by the clinical disease in man, and the correlation of these differences with the therapeutic action of different types of drugs. Because of its inconstancy, some investigators have already pointed out how exaggerated can be the importance attached to virulence in a disease as a biological character of its causal agent (Duke, 1923). The unsoundness of this basis for differentiating between species is generally admitted. However, so much is at stake that cautious students have been reluctant to merge the species, particularly in the face of discrepancies in laboratory experiences and other no

less serious difficulties in explaining some of the epidemiological discords in the human disease in relation to infection in game and cattle, etc.

After many years of intensive study of trypanosomiasis, Corson (1936) expressed the opinion that, 'As it has been shown experimentally that each of the polymorphic trypanosomes can be conveyed by more than one species of tsetse-fly, and as African natives travel so much within their own country, it does not seem likely that the peculiar geographical distribution of the trypanosomes and their association with certain tsetse-flies will be explained until at least one of the species of trypanosome has been changed experimentally into one of the other two, if that is possible. *If this could be brought about in a laboratory, no matter under what experimental conditions, it would suggest that the change might occur in nature*' (the italics are the present author's). Duke (1936), another eminent student of African trypanosomiasis, but one more inclined to be a unitarian in his views on the species-question, also admitted that 'Any acceptable theory of origin [of these two species] must be able to reconcile and explain the occurrence of the virulent and the mild types of *T. rhodesiense*.'

The experiments reported here seem to suggest the explanation sought by Duke and to fulfil the conditions demanded by Corson, for, by controlled and reproducible laboratory procedures, a typical *gambiense* trypanosome has been modified to produce a strain characteristic in every way of *T. brucei* or of *T. rhodesiense*, such as that described by Wilde and French (1945).

For purposes of convenience—if for no other reason—the agents of the human disease will doubtless continue to carry the clinical designations *gambiense* and *rhodesiense*, according to the favourable or unfavourable prognosis of the infection and as an indication for the class of drug most suitable for therapeutic purposes. If, however, it were generally recognized that these designations can carry no more taxonomic significance than the terms '*mitis*' and '*gravis*' associated with *Clostridium diphtheriae*, the nature of trypanosomiasis in man and the lower animals and the rationale for a long-range programme for its control would be immeasurably advanced.

While the implications of age of host, both human and other, as a potent element in favouring an enhancement of virulence among trypanosomes is indicated by our findings, and hints of a confirmatory nature are to be found in the general literature (cf. Chesterman, 1936, p. 301), it is clearly to be understood that it is not the intention of the writer to detract from the suggestion made by the late Professor Warrington Yorke and others to the effect that dietary deficiencies—unfortunately so prevalent among Africans—and possibly other factors (such as inadequate chemotherapy) may at times play a part in enhancing the virulence of mild (*gambiense*) strains. From this viewpoint the origin of such devastating epidemics of trypanosomiasis as depopulated the Luangwa Valley in Northern Rhodesia and certain islands on Lake Victoria can be more readily envisaged.

### SUMMARY

The low order of pathogenicity of a Belgian Congo strain of *Trypanosoma gambiense* for laboratory rodents is described. To permit the use of this strain for purposes of chemotherapeutic assays, attempts were made to enhance its virulence by various measures which have been referred to in the literature.

After such measures as rapid subinoculation by syringe through guinea-pigs, rats, mice, rabbits and hamsters, splenectomy and reticulo-endothelial system blockade had

failed to increase the virulence of the organism, recourse was taken to the inoculation of very young nursing rats.

In rats from six to 14 days old the course of infection is markedly accelerated, with development of intense parasitaemia. After the 7th serial passage of the strain through nursing rats its virulence for rats of all ages was found to be greatly augmented. It was also found to be highly virulent for mice, rabbits and hamsters, and, to a lesser extent perhaps, to guinea-pigs.

Analysis of the characters of the virulent offshoot, with special reference to its susceptibility to standard trypanocidal drugs, indicates its resemblance to a very pathogenic strain of *T. brucei*.

In the light of this transformation the taxonomic relationship between the three so-called polymorphic species of African trypanosomes is discussed. It is suggested that infection of very young hosts may represent the means whereby virulent strains of the human trypanosomes of West Africa are produced in nature.

This finding, it is thought, lends experimental support to the often-expressed opinion that *T. gambiense* and *T. rhodesiense* are mutant variants of a single species, which the international zoological laws of nomenclature would by reason of priority recognize as *T. brucei*.

*Postscript.*—The 'Nzale' strain of *T. gambiense* (or *T. brucei*, as the author would prefer to call it) and its virulent 'V' offshoot have been transferred to the Division of Tropical Medicine, National Institute of Health, Bethesda, Maryland, where they will be maintained.

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# EXPERIMENTS ON THE RELATION OF HAEMOGLOBINURIA AND ANURIA WITH REFERENCE TO BLACKWATER FEVER

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There are two main problems in blackwater fever : (i) the cause of the sudden acute haemolysis in persons infected with certain strains of malarial plasmodia, and (ii) the production of anuria in a proportion of those suffering from haemoglobinuria as a result of the haemolysis. Some evidence has recently been brought forward to show that the acute haemolysis may be due to an antigen-antibody reaction most likely to occur in those who have not yet acquired a high degree of immunity against a particular strain of *Plasmodium falciparum*, or who have lost it as a result of infrequent exposure to the bites of infected anophelines (Findlay and Markson, 1947). Three theories have been put forward to explain the anuria seen in blackwater fever.

1. Mechanical blockage (Barratt and Yorke, 1909 ; Baker and Dodds, 1925). This hypothesis does not satisfactorily account for the anuria in many cases of haemoglobinuria, since in many of those dying from anuria only a small proportion of the tubules are blocked with casts and debris, while in many patients dying from blackwater fever without anuria the debris in the renal tubules is more extensive.

2. Constriction of the renal blood-vessels supplying the glomeruli. The appearance of the kidneys in those who have died with blackwater fever associated with anuria shows that the glomerular vessels are empty (Maegraith and Findlay, 1944). Renal damage might then occur from a temporary lack of oxygen, the condition of anoxia postulated by Maegraith *et al.* (1945). Maegraith and Findlay (1944) suggested that the blood-flow through the cortex might be by-passed in anuric blackwater fever, most blood passing into the medullary vessels. A two-way renal circulation which could bring about such changes has recently been demonstrated by Trueta *et al.* (1946, 1947).

3. The nephrotoxic theory suggests that either haemoglobin itself, some derivative of haemoglobin, or some other substance may damage the kidneys so that anuria results.

The second and third theories are not necessarily opposed, since as a result of the haemolysis some substance might be liberated and either directly or indirectly produce constriction of the glomerular vessels.

Up to the present the nephrotoxic theory has had little support, for haemoglobin, as shown by Navasquez (1940), can be injected intravenously without any apparent ill effects. Mason and Mann (1931), however, demonstrated that the injection of 100 mgm. of haemoglobin into dogs caused a transient diminution in the size of the kidney, without any rise in blood-pressure. They therefore suggested that haemoglobin may have a vasoconstrictor action on the renal arterioles. In crush syndrome the kidney is in many respects similar histologically to that seen in the anuric phase of haemoglobinuria.

Eggleton (1944) was able to isolate from autolysing muscle a substance which when injected into cats produced oliguria and, later, anuria, the histological changes in the kidney being very similar to those found in the crush syndrome. The essential factor was apparently a polypeptide. Corcoran and Page (1945) gave intravenous injections of myohaemoglobin, haemoglobin and haematin to dogs whose urine had been rendered acid. They concluded that the renal damage was due to (i) tubular obstruction by pigment, (ii) impaired tubular secretory activity following the ingestion of pigment by the cells of the tubules, and (iii) the cytotoxic activity of haematin liberated within the tubules. In eight of 10 dogs in which the immediate effects of pigment injection were noted the inulin clearance was reduced by from 20 to 40 per cent. more than the diodrast clearance; in the other two dogs the inulin and diodrast clearances were reduced proportionately.

Although Corcoran and Page do not draw this conclusion, these results could be interpreted as indicating that in eight animals a differential redistribution of blood-flow had occurred in the kidney whereby the glomerular vascular beds received relatively less blood than the rest of the kidney.

## EXPERIMENTAL INVESTIGATIONS

During 1945, as part of an investigation into the problems of blackwater fever in West Africa, experiments were carried out on dogs and monkeys to determine whether haemoglobin or its derivatives might not, under certain conditions, give rise to a nephrotoxic substance. The monkeys were chiefly baboons, *Papio papio*, and green vervet monkeys, *Cercopithecus aethiops centralis*. After experimental injections animals were killed and portions of both kidneys were removed for fixation and histological examination.

### I. ESTIMATION OF GLOMERULAR FUNCTION AFTER THE INJECTION OF HAEMOGLOBIN

Dogs were catheterized, under light ether anaesthesia if necessary, and the catheter was retained in position. The dogs were then allowed to rest for one hour, after which a 10 per cent. solution of inulin was injected intravenously, the total amount injected being equivalent to 0.5 gm. per kgm. body-weight. A further period of 30 minutes' rest was then allowed. Urine collections were made over two separate periods of one hour, blood being removed in the middle of each hour. Inulin determinations were made by the method of Van Slyke *et al.* (1935). Three series of dogs were studied: a control series, a series given haemoglobin intravenously, and a series in which, before the injection of haemoglobin, the dogs had been rendered anaemic by loss of blood. Each series consisted of six dogs.

1. *Control Series.* Two inulin-clearance tests were carried out in successive hours. The average results obtained were as follows:

Inulin-clearance rate in ml. per minute	
First hour	Second hour
26	28
11	13
3	2.5

2. *Haemoglobinuric Series.* An inulin-clearance test was first carried out ; the dogs were then given haemoglobin intravenously, the usual amount being 3 gm. of crude haemoglobin suspended in 15 ml. of saline. The haemoglobin was prepared by bleeding dogs, centrifuging and repeatedly washing the red cells with physiological saline, and then haemolyzing the cells by alternately freezing them in solid carbon dioxide and thawing. A final centrifugation rendered the solution reasonably free from stroma. The dogs, when injected with haemoglobin, showed no signs of distress, and there was no diminution in the volume of urine excreted. The average results obtained were as follows :

Inulin-clearance rate in ml. per minute	
Before injection of haemoglobin	After injection of haemoglobin
23 4.5	18 3

3. *Haemoglobinuric and Anaemic Series.* In this series 100 ml. of blood was removed from a vein. Eighteen hours later the dogs appeared normal. The experimental procedure employed with the second series was then repeated. An inulin-clearance test was performed first, and then an injection of the same haemoglobin solution was given intravenously. The injection of haemoglobin into this series of dogs had a profound effect on them. All the dogs developed oliguria, and in some the urinary output diminished to nil. This decrease in the urinary output was not associated with any increase in the acidity of the urine. The average results obtained were as follows :

Inulin-clearance rate in ml. per minute	
Before injection of haemoglobin	After injection of haemoglobin
24 11 10	7 2 1

These results demonstrate that the injection of haemoglobin into a normal dog does depress to a certain slight degree the glomerular filtration-rate, if inulin clearance be assumed to measure the filtration-rate. The degree to which glomerular filtration is depressed is, however, much greater if the dogs have previously been bled. The decrease in the inulin-clearance rate might be due either to blockage of the tubules preventing the glomerular filtrate from escaping, or to a closing down of the glomerular capillary bed, leading to a failure of secretion through the glomeruli.

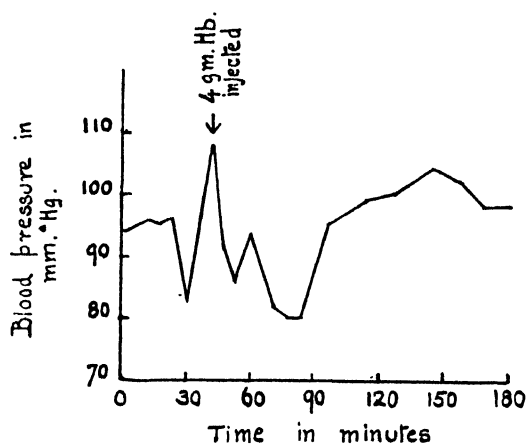
Evidence that the decrease in inulin clearance was not correlated with blockage of the renal tubules by haemoglobin debris was shown by counting cross-sections of 500 convoluted tubules in kidneys from each of the dogs in series 2 and 3, the amount of blockage being assessed as nil, slight, moderate and intense. The dogs were all killed at the end of the second inulin-clearance test. The tubular counts showed that there

was no significant difference in the extent of tubular blockage between the two series. Some dogs in series 2 showed a higher degree of blockage than the average for series 3. When the glomeruli were studied and counted, on the other hand, there was a very noticeable difference, for, whereas counts in the dogs of series 2 showed that only 32 per cent. of glomerular tufts were empty, in the dogs of series 3 the average was 78 per cent., while some dogs showed that all the glomeruli were avascular.

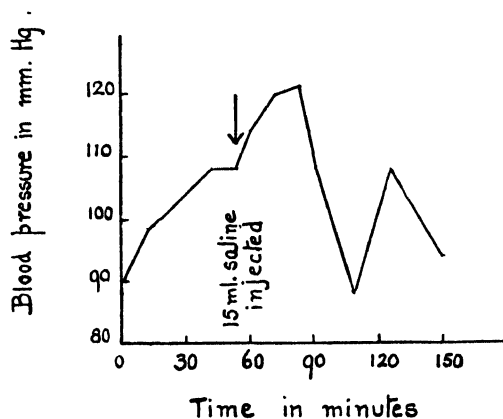
The evidence from these experiments therefore suggests that, while the injection of haemoglobin into a normal dog leads to slight impairment of glomerular filtration, this impairment is much greater when the dog has recently been subjected to haemorrhage. The reduction in the inulin clearance, together with the oliguria and anuria, can be correlated, not with blockage of the tubules, but with lack of blood-supply to the glomeruli.

## II. BLOOD-PRESSURE INVESTIGATIONS

Maegraith and Findlay (1944) suggested that in cases of blackwater fever with anuria a pronounced vascular atony developed. It was therefore thought desirable to determine what was the effect on the blood-pressure of dogs of an injection of haemoglobin. Blood-pressure were recorded on a kymograph; a mercury manometer was connected to a



GRAPH 1

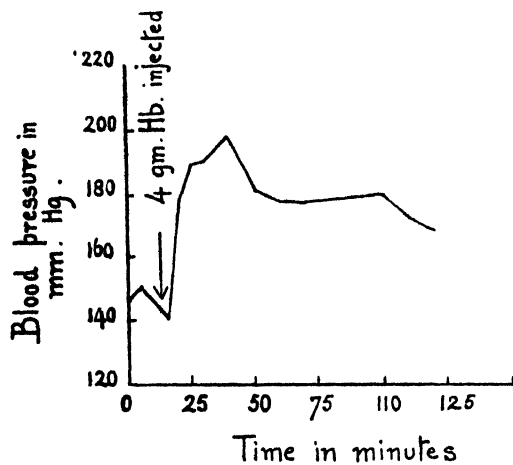


GRAPH 2

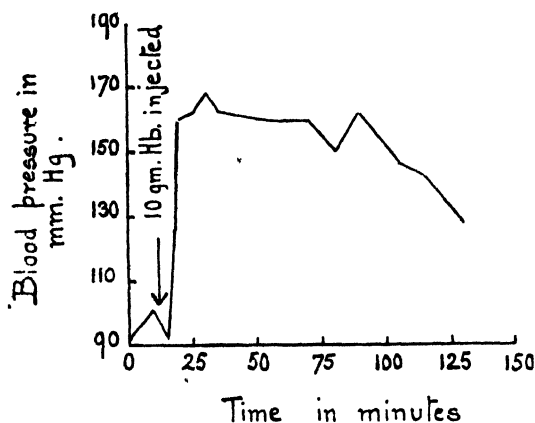
cannula in the dog's femoral artery, and sodium citrate was used as an anticoagulant. The operative procedure was carried out under ether, the anaesthesia being maintained with somnifaine. The haemoglobin solution was freshly prepared, as in the previous experiments.

*Series A.* Four dogs were bled, 100. ml. of blood being removed; 18 hours later the blood-pressure was determined. Three dogs were then injected intravenously with 4 gm. of haemoglobin in 15 ml. of saline, a control animal being given 15 ml. of physiological saline. No special changes were noted in the blood-pressures either in the dogs receiving haemoglobin or in that receiving saline (graphs 1 and 2). There was no tendency for any rise of blood-pressure to occur after injection, nor for the pressure to fall below its resting value.

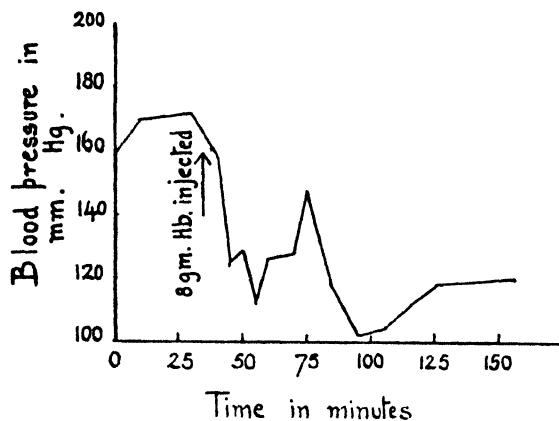
*Series B.* A second series of 20 dogs were bled as before, 100 ml. of blood being removed 18 hours before further investigations were made. Thirty minutes before the blood-pressure recordings were begun both carotids were ligated. Under these conditions the dogs lay quite still and no anaesthesia was necessary. After the resting blood-pressure had been determined haemoglobin was injected intravenously. It was hoped that by this operative procedure the animals would show more clearly any response to the presence



GRAPH 3



GRAPH 4



GRAPH 5

of a hypothetical pressor substance. This hope was fulfilled, as all the dogs showed a well-marked rise in blood-pressure. Two typical blood-pressure recordings are shown in graphs 3 and 4.

*Series C.* A control series of six dogs was not bled, but all the dogs had bilateral carotid ligation performed 30 minutes before the blood-pressure readings were begun. Despite the injection of haemoglobin, no dog showed a pressor response (graph 5).

Histological examination of the kidneys of dogs in the three series showed, as before, a variable amount of debris in the lumen of the tubules, while in the animals of series A and B the glomerular capillaries were for the most part empty.

It was thus demonstrated that unbled dogs, despite ligation of the carotid arteries and despite the injection of haemoglobin, do not exhibit any characteristic rise of pressure, nor from the first experiments on inulin clearance does the injection of haemoglobin in the unbled animal cause marked decrease in glomerular function. In the case of the dogs bled before injection of haemoglobin, there was diminution of glomerular filtration, and when the carotids were ligatured a rise in systemic blood-pressure could be demonstrated: this could be correlated with absence of blood from the glomerular capillaries.

It may be noted in passing that patients with blackwater fever rarely show any fall in blood-pressure until immediately before death, even though they are losing considerable quantities of red blood-cells as the result of haemolysis.

#### *The Demonstration of a Pressor Substance in the Blood*

The pressor response which has been noted in bled dogs injected with haemoglobin might be due either to a nervous mechanism or to the presence of a pressor substance in the plasma. If the pressure effect were dependent on a humoral agent, then it should be possible to demonstrate it in the blood of those dogs which have shown a pressor response to haemoglobin, while it should be absent from the blood of those animals in which no such response had followed the injection of haemoglobin.

If the hypothetical pressor substance were, as seemed likely, a break-down product of haemoglobin, then it would possibly be heat-stable. Preparation of the plasma for demonstration of the pressor substance was carried out on this hypothesis. Five ml. of blood was withdrawn from the dogs before and at intervals after the injection of haemoglobin. The blood was mixed with sodium citrate and centrifuged, and the plasma was pipetted off. The plasma was then heated in a water-bath maintained at 90° C. for 30 minutes to precipitate the plasma proteins. The resulting gel was mashed up in 1 ml. of physiological saline, the mixture centrifuged, and the clear supernatant fluid pipetted off. This fluid was used for testing for the presence of pressor substance. The estimation of pressor activity was carried out on spinal monkeys, with a mercury manometer connected to a cannula inserted into the femoral artery. One ml. of saline extract containing the hypothetical pressor substance was injected intravenously into the monkey. The maximum rise in blood-pressure was then noted. The results of experiments on three dogs given haemoglobin intravenously 18 hours after being bled is shown in the table below.

It will be seen that from 60 to 90 minutes after the injection of bled dogs with haemoglobin a pressor substance could be demonstrated in the plasma by its effect on the blood-pressure in spinal monkeys. This corresponds to the period when, as shown in graphs 2, 3 and 4, there was a rise in the blood-pressure of the bled dogs following injection of haemoglobin. Three control dogs, which were given similar injections of haemoglobin but were not bled previously, at no time yielded any pressor substance from their plasma, as shown by injection of spinal monkeys.

The main conclusions which can be drawn from these experiments are as follows:

1. When the haemoglobin is injected into an anaemic animal a substance is produced which diminishes the glomerular filtration-rate by shutting off what Trueta and his colleagues (1947) have termed the lesser circulation in the kidney.

## The pressor activity of plasma from dogs bled and given haemoglobin intravenously

Time, in minutes, after haemoglobin injection when blood was removed	Maximum rise of blood-pressure (in mm. Hg.) in spinal monkeys following injection of dog-plasma extract		
	Dog 1	Dog 2	Dog 3
0	0	0	0
5	16	0	—
10	—	—	0
20	0	—	—
25	—	0	0
30	—	—	—
40	0	—	—
45	—	0	0
60	16	—	—
65	—	14	—
70	—	—	7
85	—	12	—
90	8	—	0
105	—	0	—

2. The pressor substance is to be found circulating in the blood-stream and is capable of causing a generalized rise in blood-pressure.

3. The production of this pressor substance depends in some way on diminished oxygen-carrying power of the blood. Page (1943) demonstrated the presence, after severe haemorrhage, of a vasoconstrictor substance in the plasma; the formation of the pressor substance occurred as a result of anoxia of the tissues.

### III. INVESTIGATIONS IN CASES OF HUMAN HAEMOGLOBINURIA

A chance observation demonstrated that, when a pregnant woman with a mild toxæmia of pregnancy was given a blood transfusion, the histidine output in the urine for the 24 hours after the transfusion was increased. It was therefore thought that in cases of haemoglobinuria, following an acute intravascular haemolysis, the output of histidine would also be found to be increased.

The following investigations were carried out on cases of haemoglobinuria in Africans in a large Army General Hospital in West Africa.

1. Whenever the patient passed urine the time and volume were noted and a specimen was saved for laboratory investigation.

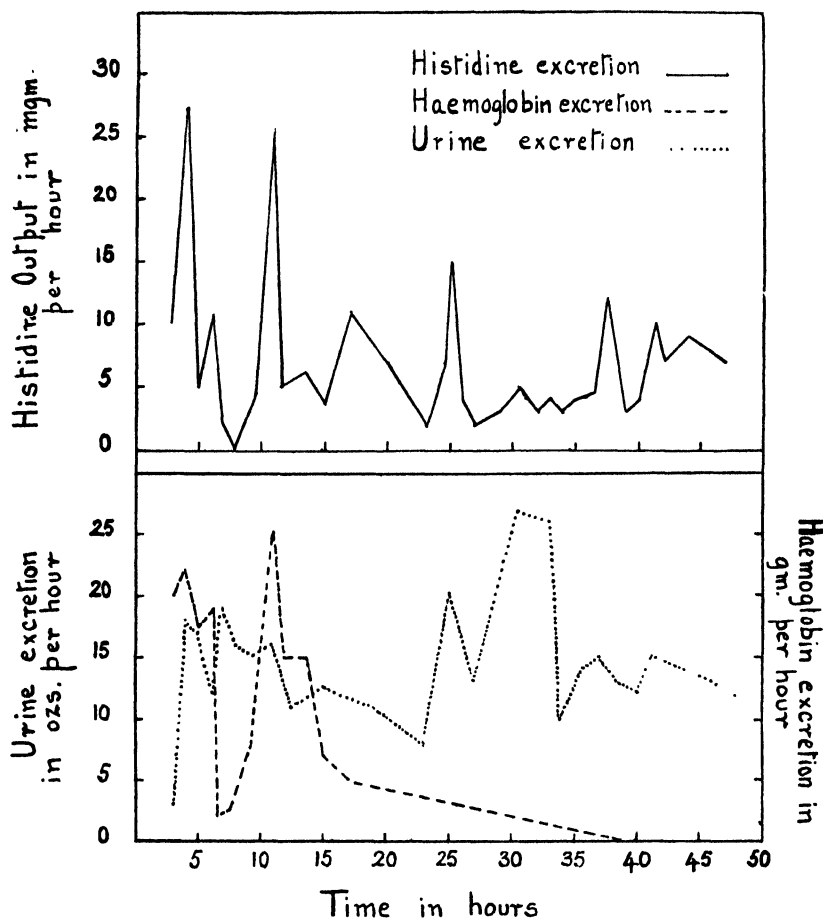
2. Histidine determinations were carried out on each specimen saved.

3. The haemoglobin content of each specimen of urine was estimated by converting the haemoglobin to acid haematin and then extracting with ether. The resulting solution was estimated against a standard solution of acid haematin in ether.

Graphs 6 and 7 show the relationship between the volume of urine excreted, the haemoglobin loss in the urine, and the histidine excretion. The curves show that as long as the urinary output is well maintained there is good correlation with the output both of haemoglobin and of histidine. In graph 6, where the histidine output was maintained at a high level after the haemoglobinuria had ceased, the patient was passing through an acute sickling crisis. (Robertson and Findlay, 1947), and, though the continuing haemolysis was insufficient to cause the excretion of haemoglobin in the urine, there was

evidence that *in vivo* sickling was still occurring. The patient in graph 7 was suffering from blackwater fever.

Graphs 8 and 9 are also from patients with blackwater fever. In these two cases the urinary output diminished during the course of the hæmoglobinuria, though anuria did not occur. Both graphs show a lack of correlation between the output of haemoglobin and that of histidine.

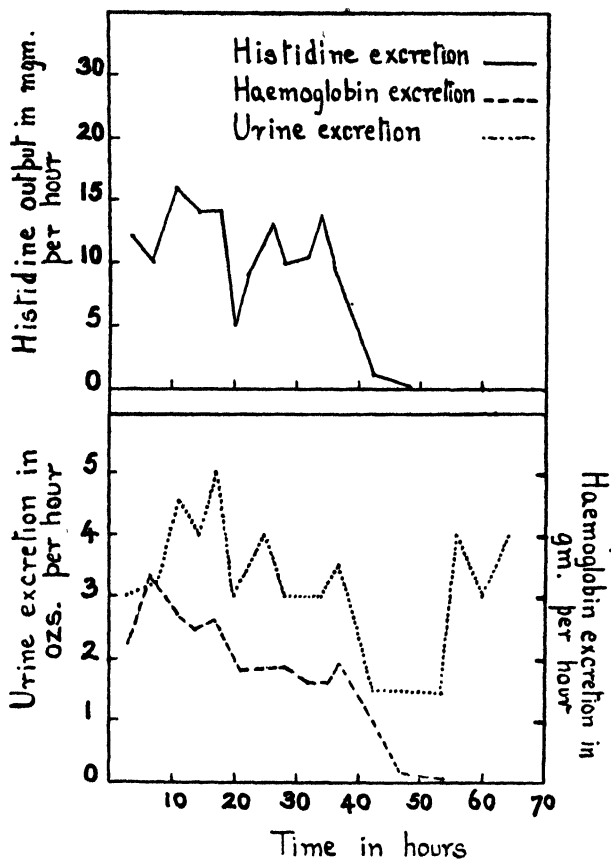


GRAPH 6

The possible explanations for this discrepancy are: (i) that histidine retention occurred as a result of the failure of urinary output; (ii) that histidine was not being produced.

Some support for the latter suggestion is found in the work of Kapeller-Adler (1944), who showed that in patients with toxæmia of pregnancy the urinary output may be within normal limits, but the histidine output is minimal, owing to its conversion to histamine. Incidentally, the kidneys in patients dying from eclamptic anuria present a histological picture very similar to that seen in the anuria of blackwater fever.

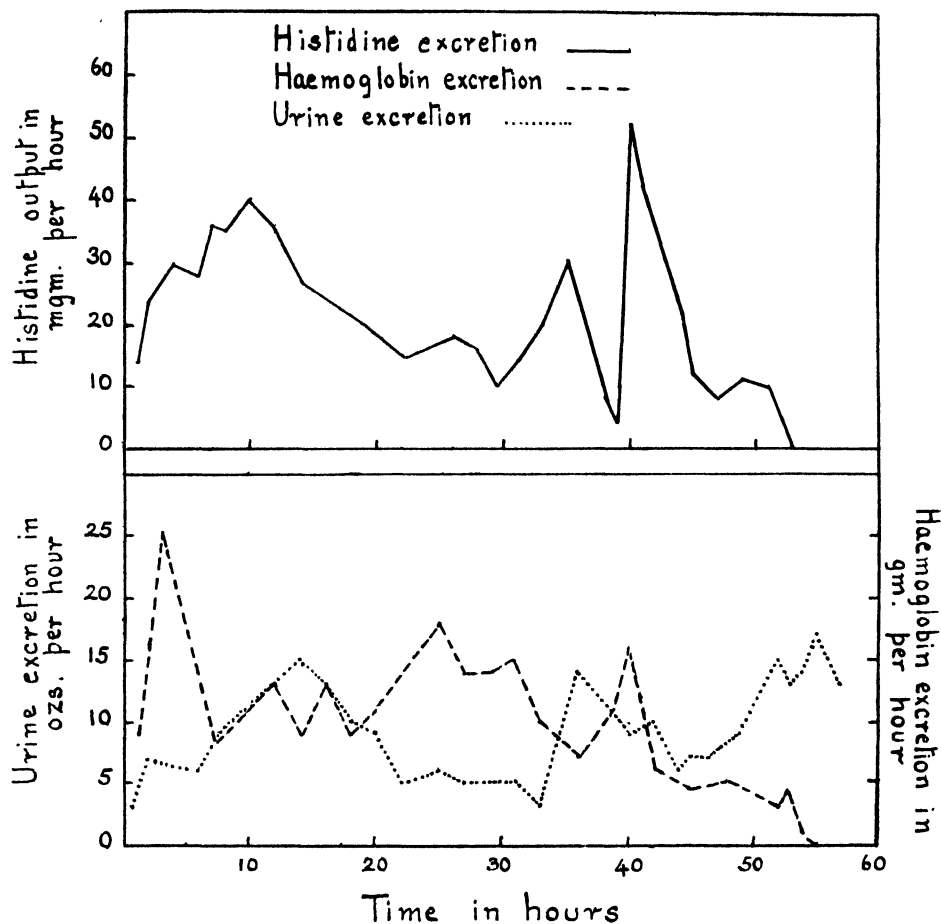




GRAPH 7

Further light was shed on the problem by the study of a case of haemoglobinuria with anuria. The patient, when first seen, had had haemoglobinuria for three days ; he was then very anaemic, and his urinary output had greatly diminished. Urine was drawn off by catheter ; otherwise no urine was passed normally. On the third day of this oliguric phase the patient died, with a grossly elevated blood-urea. The specimens of urine obtained were dark black in colour, owing to the presence of haemoglobin and allied pigments. The following are the results obtained for histidine and urine excretion :

Date	Time	Volume of urine in ml.	Histidine per 100 ml.
1.7.45	13.50 hours	4	20
2.7.45	11.00 hours	30	10
3.7.45	13.00 hours	25	0

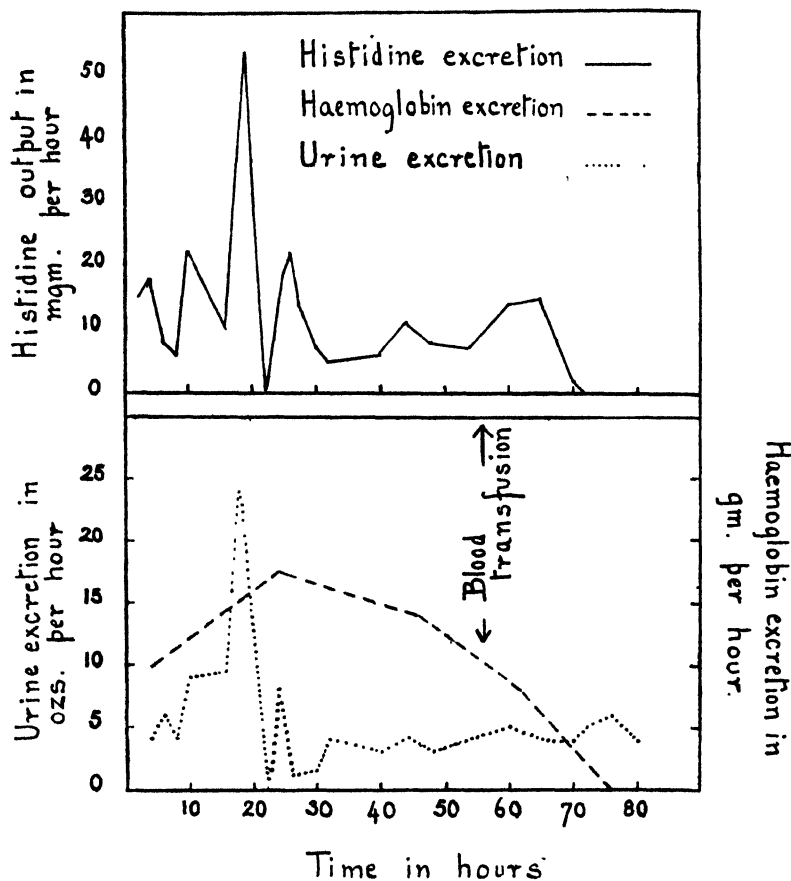


GRAPH 8

The patient died shortly after the last specimen of urine had been removed. A necropsy was carried out immediately after death and 200 ml. of blood were obtained. The serum was separated off and heated for 30 minutes in a water-bath at 90° C., and the protein gel was extracted with a small amount of saline. The extract was centrifuged and tested for pressor activity in a spinal monkey as in the previous experiment. The following results were obtained :

Amount of extract injected	Maximum pressor response in mm. Hg.
2 ml.	8
4 ml.	16
6 ml.	32

It is now possible to draw a parallel between the animal experiments and cases of haemoglobinuria in man. In the unbled dogs injections of haemoglobin produced little effect on renal function, no rise in blood-pressure, and no pressor activity in the plasma. Similarly, in cases of haemoglobinuria in man haemoglobin can be passed quite freely, with a minimal disturbance of renal function, and no pressor activity is found in the serum. In bled dogs injections of haemoglobin produced marked alteration in renal function, and pressor activity could be demonstrated in the plasma of such animals.



GRAPH 9

Similarly, in the human case of haemoglobinuria with a profound alteration in renal function, a pressor substance was present in the serum. In addition, it would appear that histidine retention in the last type of case is in some way related to the renal failure and the development of pressor activity in the serum.

Page and Helmer (1940) described the preparation of a pressor substance, angiotonin, from a substrate of a globulin and the enzyme renin. Renin is believed to be liberated from kidneys which are rendered anoxaemic. The pressor substance is a polypeptide and contains the histidine fraction of the globulin. The suggestion is therefore made that some similar break-down of haemoglobin occurs in cases where the kidneys have been

rendered anoxaemic, and that histidine retention occurs because the histidine is locked in this pressor substance. A similar substance to angiotonin, pepsitensin, has been prepared by the action of pepsin on a globulin. Pepsitensin substance may not be identical with angiotonin (Plentl and Page, 1944), but it also is rich in histidine.

Attempts were therefore made to prepare pressor substances similar to angiotonin and pepsitensin, using haemoglobin as the substrate.

*Preparation of Pepsitensin.* Washed human red cells were obtained from 500 ml. of blood. Commercial pepsin had to be used as no facilities existed for the preparation of the crystalline variety. The pH of the red cells was adjusted to approximately 5, and a suspension of 1 gm. pepsin was added. Incubation was at 32° C. for 10 minutes. At the end of that time the mixture was heated for 30 minutes at 90° C. The precipitate was filtered off and the filtrate centrifuged. The clear supernatant was used for determining pressor activity after the pH had been brought back to 7. Spinal monkeys were again used.

Amount injected, in ml.	Maximum rise of blood-pressure, in mm. Hg
Extract 1 : 1	11
2	7
Extract 2 : 1	13
2	25
Extract 3 : 1	14
2	14

Pressor activity was not always found in the extract. If an extract showed good pressor activity, it had a low free histidine content, and when the extract was rich in histidine no pressor activity was found. Thus, extract 3 had from 0 to 3 mgm. of free histidine per 100 ml., while extracts 1 and 2 contained 20-40 mgm. of free histidine per 100 ml.

*Preparation of Angiotonin.* Dried defatted kidney powder was prepared. Following the method of Plentl and Page (1944) an extract of the powder was prepared. This extract is believed to be free from angiotonase activity. Using this extract, angiotonin was prepared according to the above method, except that haemoglobin was used as the substrate. The results were unsatisfactory, and in only one case was a final product prepared which showed pressor activity when injected into spinal monkeys.

Amount injected, in ml.	Maximum blood-pressure rise, in mm. Hg.
3	6
5	12

Working under tropical conditions may have interfered with the production of an active renin preparation, but Page and Helmer (1940) were unable to produce a pressor substance from haemoglobin using renin as the enzyme. The fact that pepsin appears to be able to split haemoglobin to produce a pressor substance does make it likely that an enzyme similar to pepsin may exist *in vivo* and that it will give rise to a pressor substance from haemoglobin.

## DISCUSSION

From the results presented it is possible to suggest a general picture of the pathological processes which take place in the kidney following an intravascular haemolysis.

Coincidental with the haemolysis a progressive anaemia develops. This may be sufficient to cause a mild degree of anoxia of the kidney. The degree of anoxia will depend on the rapidity and extent of the haemolysis and on the blood picture of the patient before the haemolysis occurred.

The increased histidine excretion in the urine following the haemolysis would appear to be due to break-down of haemoglobin, for globin contains about 10 per cent. of histidine. Even in those cases, however, where the histidine excretion appears to be maximal, the amount excreted corresponds to only a small fraction of the total haemoglobin liberated. It is in these cases that the histidine excretion closely follows the haemoglobin excretion, and in these cases the kidneys continue to function with little demonstrable impairment. If the histidine excretion diminishes and haemolysis is still going on, the patient develops renal failure which may progress to death. The failure of histidine excretion we believe to be due to the development of a pressor substance in the blood. This pressor substance is developed from globin and is probably a polypeptide containing all the histidine present in globin. The production of this pressor agent depends on renal anoxia, and, by analogy with the renin-angiotonin system, renal anoxia would produce an enzyme capable of splitting globin to the polypeptide level. The process is temporarily halted at this stage. This pressor agent will constrict the glomerular arterioles, tend to increase the degree of renal anoxia, and diminish the glomerular filtration-rate. An increase in renal anoxia will further increase the production of the enzyme capable of producing the pressor agent from globin. A pressor substance has been demonstrated, but conclusive proof does not exist for the hypothesis which suggests that renin is the enzyme responsible for its production.

If, according to Maegraith *et al.* (1945), a nephrotoxic theory is to explain satisfactorily the renal changes in anuria associated with blackwater fever, it must also provide some explanation for the 'renal syndrome' seen in other conditions. Similar renal changes are seen in the following conditions:

1. Incompatible blood transfusions.
2. Sickle-cell crisis with haemoglobinuria.
3. Concealed accidental haemorrhage in pregnancy.
4. Crush syndrome.
5. In animals, following the temporary constriction of the renal arteries.
6. Yellow fever.
7. Cholera.
8. *Clostridium welchii* infections.
9. Leptospiral jaundice.
10. Burns.

In the first three conditions, as well as in blackwater fever, the pressor substance could be formed by the break-down of haemoglobin. Crush syndrome has probably rather a different aetiology, since Eggleton (1944) found that the toxic agent is a polypeptide formed in the muscle whilst it was compressed and anoxaemic. The fifth condition is likely to produce renin from the temporarily anoxaemic kidney, and this will produce angiotonin, a nephrotoxic agent. In burns Page (1943) noted the presence of a vasocon-

strictor substance in the blood. In yellow fever, cholera and the other conditions there is a profound toxæmia, with tissue necrosis.

The general principle of proteolytic enzymes being liberated from tissues under anoxic conditions, and acting on various protein substrates to produce nephrotoxic polypeptides, appears to be possible in all these cases.

Such a hypothesis does not exclude the fact that a nervous mechanism may also play a part in producing anoxæmia of the glomeruli. O'Connor and Verney (1945), for instance, have noted the sudden cessation of diuresis in dogs submitted to emotional stress. Similarly, among 112 cases of blackwater fever in African soldiers oliguria and complete anuria were extremely rare, while among European soldiers with blackwater fever they were by no means uncommon. The European attacked with blackwater fever was apprehensive and fully alive to the dangers of his condition; the African was placid and entirely unconscious of the complications which might so easily prove fatal.

### CONCLUSIONS

Intravenous injection of haemoglobin in dogs caused little or no change in the inulin-excretion rate in the urine. If the dogs had been bled 18 hours previously the inulin-excretion rate was decreased; oliguria and even anuria developed. Histological examination of the kidneys of such dogs showed that in bled dogs injected with haemoglobin the glomeruli were bloodless.

The injection of bled dogs with haemoglobin causes a rise in blood-pressure, which appears to be due to the presence of a pressor substance in the plasma.

In patients with haemoglobinuria there is an increased excretion of histidine in the urine, but in patients with urinary failure the excretion of histidine falls.

A patient dying with anuria associated with haemoglobinuria was found to have a pressor substance in the serum at death.

Preparation of pepsitensin from red blood-cells showed that extracts with good pressor activity had a low free histidine content, but when the extract was rich in histidine no pressor activity was found.

Evidence is brought forward to suggest that retention of histidine may be related to renal failure and to the development of pressor activity in the blood.

It is suggested that in all cases of renal anoxia a proteolytic enzyme is liberated from the tissues. This enzyme acts on various protein substrates to produce polypeptides, which act as constrictors of the glomerular arterioles and thus decrease the blood-supply to the glomeruli.

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# MALIGNANT DISEASE IN NIGERIA : AN ANALYSIS OF A THOUSAND TUMOURS

BY

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AND

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An analysis of malignant tumours in natives of Nigeria was published by Smith and Elmes in 1934. The present paper is a continuation of that work, and the tumours here analysed represent specimens received from all parts of Nigeria during the 10 years 1935-44. All were from Africans.

Accurate statistics are still difficult to obtain, and the general remarks by Smith and Elmes (1934) concerning the difficulty in obtaining accurate data apply equally to this analysis.

In the light of more recent knowledge, some of the tumours have been reclassified, with consequent alteration in the percentage incidence.

The classification is according to the varieties of tumour, the age and sex incidence, and the type and site incidence of the two main groups, carcinoma and sarcoma.

The difficulty in obtaining photographic materials during and after the recent war, and the high cost of reproduction, preclude photomicrographs, except for two tumours of special interest—a pigmented acanthoma and the chronic leg ulcer type of carcinoma.

TABLE I  
Varieties of tumours

Variety	No.	Percentage
Carcinoma ... ..	568	56·8
Sarcoma ... ..	204	20·4
Melanoma ... ..	62	6·2
Mixed salivary-gland tumour ...	49	4·9
Neuroblastoma ... ..	45	4·5
Osteoclastoma ... ..	23	2·3
Adamantinoma ... ..	18	1·8
Haemangio-endothelioma ... ..	10	1·0
Ewing's tumour of bone ... ..	8	0·8
Granulosa-cell tumour of ovary ...	4	0·4
Brenner's tumour of ovary ... ..	3	0·3
Teratoma ... ..	3	0·3
Carotid body tumour ... ..	2	0·2
Lympho-epithelioma of nasopharynx	1	0·1
Total ... ..	1,000	100

## NOTES ON TABLE I

*Haemangio-Endothelioma.* It is interesting to record that one of these tumours, in the lip of a boy of 11 years, gave rise to fatal haemorrhage.



*Neuroblastoma.* These growths were included under 'round-cell sarcoma' by Smith and Elmes (1934). We have here separated them by virtue of their histology, their site of origin, and, to a less extent, their age incidence. They include the undifferentiated sympathicoblastoma, the retinoblastoma and the medulloblastoma.

Of the 45 cases in the present analysis, 29 were in males, 11 were in females, and in five the sex was not stated. The youngest case was a boy of  $1\frac{1}{2}$  years, with a retinoblastoma; the oldest was a man of 50, with an abdominal sympathicoblastoma.

Thirty-one of the patients were under 10 years of age, six were between 10 and 20 years, and only one was above the age of 20. The age was not stated in three instances.

Twenty-one of the specimens were retinoblastomas, 16 were abdominal sympathicoblastomas, three were medulloblastomas, three were from the maxilla, one from the temporal bone, and one from the scrotum.

*Melanoma.* These number 62 in the present analysis. The percentage of 6.2 agrees fairly closely with the 8.0 per cent. obtained by Smith and Elmes (1934), and with the 10.8 per cent. obtained by Vint (1935) in East Africa.

Ewing (1940) quotes Bauer on the rarity of melanotic tumours in the negro, but their frequent occurrence in the natives of the Anglo-Egyptian Sudan is recorded by Hewer (1935), who stresses the different conditions under which American negroes live.

That trauma plays a significant part in the aetiology is borne out by the site incidence of our cases. Forty-five (72.0 per cent.) occurred on the foot, four of them with metastases in the inguinal lymph-nodes. Of the remainder, four were in the mouth—one of which had widespread metastases—four were in the eye, and there was one each in wrist, buttock, scalp and femoral triangle. Two were described briefly as 'ulcers' and were probably of the lower limb, and three were metastatic growths with undetermined primary sites.

Thirty-one of the melanomas were in males, 17 were in females, and in 14 the sex was not stated. In only 32 cases was the age given. There were two tumours in boys of eight years, both situated in the eye. The remainder occurred between the ages of 25 and 65. Of the 32 cases in which the age was not recorded, 18 were stated to be adults.

Histologically, the tumours were, for the most part, of the spindle-cell (sarcomatous) or polyhedral (carcinomatous) type, the amount of pigment usually being abundant but varying from microscopic amounts to coal-black masses. One specimen deserves special comment: it occurred in the eye of a man of 30 years and was structurally a pigmented acanthoma with well-marked 'cell nests' (see Plate XII). No previous record of such a tumour could be found in the available literature.

With regard to the aggressive character of the melanomas, there is some doubt whether this is as great in the dark-skinned African as it is in the light-skinned races, but the difficulty of following up operation-cases rules out accurate statistical evidence. Eight out of the 62 cases were metastatic tumours or had given rise to metastases, all with abundant pigment. Hewer (1935) in the Sudan found metastases in the lymph-nodes in 10 of his 47 cases.

*Adamantinoma.* All the tumours, except one, occurred in the jaw. The exception was in the tibia and has been recorded by Bell (1942).

*Teratoma.* Only those with definite histological evidence of malignancy are included in this analysis. Two occurred in the ovary and one in the sacro-coccygeal region. Since the analysis was completed one specimen of testicular teratoma has been received.

*Osteoclastoma.* These tumours were classified by Smith and Elmes (1934) as myeloid sarcoma. The present analysis includes the bone tumours, the giant-cell epulis and the giant-cell tumour of tendon sheaths. Nine occurred in bones (radius, tibia, rib), nine in tendon sheaths, and five were of the epulis type. One of the epulis tumours, in a man aged 35 years, was remarkable in that it reached the size of an infant's head.

*Ewing's Tumour of Bone.* Only one of the eight cases came to autopsy and thus allowed confirmation of the diagnosis, but in the others the clinical examination failed to reveal a primary carcinoma or neuroblastoma, both of which may, in their metastases, closely simulate Ewing's tumour. The sites involved were the ilium, clavicle, leg bones, forearm bones, scapula and os calcis. The tumour of the os calcis had secondary deposits in the regional lymph-nodes.

Five of the tumours were in males, two were in females, and in one the sex was not stated. Five occurred in persons under the age of 30 and none above the age of 45.

TABLE II  
Age and sex incidence

Age-group ...	0-10 years	11-30 years	Over 30 years	Not stated	Totals
Males ...	35	114	308	22	479
Females ...	24	60	217	59	360
Not stated ...	8	7	19	127	161
Totals ...	67	181	544	208	1,000

#### NOTES ON TABLE II

Malignant tumours occurring under the age of 10 years show almost the same percentage in the two analyses—5·8 per cent. in the earlier one of Smith and Elmes (1934), and 6·7 in the present one. The difference between the sexes increases sharply after the age of 10, and is mainly accounted for by the greater frequency with which males attend hospitals. (In the 10-year period up to 1943 the African in-patients in Government hospitals numbered 600,685, of whom 71·5 per cent. were male and 28·5 per cent. female.)

#### NOTES ON TABLES III AND IV

The term 'glandular carcinoma' includes all carcinomas arising in glandular organs and tissues, and all in which the histology is of a definite glandular type. 'Undifferentiated carcinoma' is one in which the histological type shows no differentiation into glandular structure and where there is no definite indication of origin. The embryonic carcinomas comprise nine choriocarcinomas, seven seminomas, two dysgerminomas and four neuroblastomas (Wilms' tumour).

*Kidney.* No attempt has been made to distinguish between hypernephroma and adenocarcinoma, as such distinction is felt to be no longer valid.

*Female Genitalia.* Seven carcinomas occurred in the vulva, five in the vagina, 68 in the uterus, and 20 in the ovary. Owing to meagre clinical data it was not possible to subdivide the uterine cancers into cervix and corpus. The great majority occurred in parous women and they include the nine choriocarcinomas.

The ovarian carcinomas include the dysgerminomas, both of which occurred in children.

*Male Genitalia.* There were five squamous carcinomas of the scrotum and 10 of the penis. The seven testicular tumours were all seminomas. The prostatic carcinomas numbered eight.

TABLE III  
Carcinoma : type incidence

Type	No.	Percentage of carcinomas
Glandular ... ..	243	42.8
Squamous ... ..	231	40.7
Basal-cell ... ..	16	2.8
Undifferentiated ... ..	56	9.8
Embryonic ... ..	22	3.9
Total ... ..	568	100.0

TABLE IV  
Carcinoma : site incidence

Site	No.	Percentage of carcinomas
Female breast ... ..	77	13.5
Male breast ... ..	7	1.2
Kidney ... ..	10	1.8
Female genitalia ... ..	100	17.6
Male genitalia ... ..	30	5.3
Liver, primary ... ..	81	14.3
Liver, metastatic ... ..	3	0.5
Skin ... ..	122	21.5
Thyroid ... ..	14	2.5
Stomach ... ..	22	3.9
Lip, mouth, palate, tongue, larynx ...	13	2.3
Nasal cavities ... ..	6	1.1
Bladder ... ..	18	3.1
Pancreas ... ..	7	1.2
Lung ... ..	5	0.9
Large intestine ... ..	14	2.5
Lymph-nodes, metastatic ... ..	16	2.8
Salivary glands ... ..	11	1.8
Bone, metastatic ... ..	6	1.1
Small intestine ... ..	1	0.2
Unspecified ... ..	5	0.9
Total ... ..	568	100.0

*Liver.* All but one of the 85 tumours of the liver were carcinomas. Of these, 81 were primary, which is 8.1 per cent. of the total tumours in this analysis and corresponds fairly closely to Smith and Elmes's (1934) figure of 6.4 per cent. of all tumours. The 81 primary carcinomas represent 14.3 per cent. of the total carcinomas, which is close to Vint's (1935) figure of 13.0 per cent. in East African natives. Much higher percentages have been recorded by some writers, and Kennaway (1944), quoting Berman, gives liver

cancer as 37·4 per cent. of all cancers in the Bantu races and 18·7 per cent. in West Africans, and draws attention to the much lower incidence in American negroes. In the present analysis 70 of the primary growths originated from hepatic cells and 11 from the intrahepatic bile-ducts. All showed cirrhosis of the Laënnec type to a greater or less degree, a process which Ewing (1940) considers is contributory to and coincident with the tumour growth. When the high incidence of liver damage and consequent cirrhosis in Africans and other tropical races is explained, we shall also have an explanation for the high percentage of primary liver cancer. It is unlikely that alcohol, syphilis, schistosomiasis, virus disease or race *per se* will be specially incriminated, though they may be contributory to damage caused by a common initial factor. Des Ligneris (1936), discussing the incidence of liver cancer in young mine natives in South Africa, considers that the factor responsible for cirrhosis must be one affecting them in their early youth.

Smith (1942) draws attention to the abnormal histological appearance of the liver in Nigerian natives, and notes the frequency with which subacute necrosis is encountered. A dietetic factor seems the most likely initiator of liver damage, and is suggested by the work of Gillman (1944) and of Himsworth and Glynn (1944).

In the present analysis 19 of the cases were under the age of 40, the youngest being a man of 20. Twenty-eight were between 40 and 60 years and five were between 60 and 70. Sixteen were stated in the clinical reports to be adults, and in 17 cases no age was given.

Clinical data are frequently meagre, but of the 60 cases in which the sex was given 57 were males. One must bear in mind the much greater proportion of males attending hospitals, but there does seem to be a true preponderance of liver cancer in the male sex.

Primary carcinoma of the liver in natives of Nigeria is a disease seen, as a rule, only in its final stage, and it is remarkable how, with the organ grossly diseased, the individual manages to perform his vocation. A recent case seen by one of us (B.G.T.E.) was a soldier in whom disease was unsuspected until his admission to hospital a week before death. At autopsy the liver weighed 11 pounds and was almost entirely composed of tumour tissue.

*Skin.* Squamous carcinoma of the leg merits special mention in this paper. Vint (1935) has drawn attention to the prevalence of malignant change in chronic tropical ulcers of the leg, and stresses the difficulty in determining whether precancerous changes exist in cases with aberrant overgrowth of the marginal epithelium. Cooray (1944), in Ceylon, found that 61 per cent. of the skin cancers arose in connection with chronic ulcers of the leg. We have seen several cases of chronic leg ulcer with a distinctive clinical appearance. The base is composed of granulation-tissue and the edge is nodular, indurated, raised and rolled, and very suggestive of squamous carcinoma. Histologically, there is marked papillary hyperplasia of the epithelium and the formation of 'cell nests.' Mitoses, hyperchromatism and abnormal cell-bodies or nuclei are rare, and the down-growth appears to be limited, as Vint (1935) notes, by the fibrous tissue of the ulcer bed. To these tumours we have given the name 'squamous carcinoma of the chronic leg ulcer type,' in the hope that it will connote a definite entity to the clinician.

Twelve of the squamous carcinomas of the series were of this type, and the majority were of several years' duration, without evidence of metastases. Seven were in males, four were in females, and in one the sex was not stated. The youngest patient was 28 years of age and the oldest 63. In the few cases which we have been able to examine

clinically, no evidence of a fuso-spirochaetal infection was found, though there remains the possibility that they were primarily true examples of tropical ulcer. Enlarged regional lymph-nodes showed, on section, only chronic inflammatory changes. Excision and skin grafts were the normal methods employed in treatment.

Dr. E. C. Braithwaite, Senior Specialist, Nigeria, in a personal communication on the subject of these tumours states: 'In these cases of malignant disease (epithelioma) arising in ulcers of long standing, particularly burn cases, the malignancy is of a low grade, with late secondary involvement of the lymphatic glands in the drainage area. Death is usually due to some intercurrent disease.'

Plate XIII shows a typical squamous carcinoma of this type in a man aged 30 years with a history of an ulcer of the leg of 10 years' duration.

**Stomach.** Twenty-two cases of carcinoma of the stomach occur in the present analysis, compared with only four cases in the first analysis by Smith and Elmes (1934). Vint (1935) recorded an incidence of 1.8 per cent. in his malignant tumours in Kenya, which is close to our figure of 2.2 per cent. Cooray (1944), in Ceylon, found only seven primary carcinomas of the stomach in 2,295 malignant tumours. These figures cannot strictly be compared with the high percentages quoted by Ewing (1940) for Europe and the United States of America, but there may be, as Ewing notes, a slight relative immunity from this form of cancer in negroes and in the tropics. No reliable statistical evidence is likely to be obtained from Nigeria for many years.

Thirteen of our cases were in males, five were in females, and in four the sex was not stated. The 16 cases in which the age was given were between 30 and 60 years.

Histologically, all were adenocarcinomas, except two, one which was carcinoma simplex of diffuse type and the other a sclerosing fibrocarcinoma.

**Bladder.** The only point of interest is that, out of 18 carcinomas, four were associated with *Schistosoma haematobium* infection.

#### NOTES ON TABLES V AND VI

In the analysis by Smith and Elmes (1934) the sarcomas were 44.0 per cent. of the total, compared with 20.4 per cent. in the present series. This difference is largely made up by the neuroblastomas, melanomas, osteoclastomas and Ewing's tumours, which have been separated from the sarcomas in this paper.

**Kaposi Sarcoma (Multiple Idiopathic Haemorrhagic Sarcoma).** This interesting condition is by no means rare in the natives of Nigeria, and 24 (2.4 per cent.) of the present analysis were of this type. The earlier analysis by Smith and Elmes (1934) showed a corresponding figure—2.0 per cent.

With two exceptions, in which the sex was not stated, all the cases occurred in males, the youngest being 23 years of age and the oldest 50. The tumours were confined to the leg below the knee, except for two cases in which the external genitals, perineum and thighs were involved, and one case in which the left upper arm, as well as the leg, was affected. No metastatic deposits are recorded in the clinical data, nor were any detected in the few cases observed personally. If the disease is a granuloma of peculiar type we have not been fortunate to see a case in which the histological picture was otherwise than predominantly neoplastic. The earliest nodule was probably not more than six weeks old, but the structure was that of angiosarcoma. Only in cases with ulcerated nodules were inflammatory cells at all numerous.

An unsuccessful attempt to transmit the disease to experimental animals by one of us (B.G.T.E.) in conjunction with Dr. G. M. Findlay has been recorded by Findlay (1946). In addition to the chimpanzee, baboons, *Cercopithecus* and Patas monkeys, rabbits, guinea-pigs and mice, two Syrian hamsters were also inoculated without success. The nodules used for the inoculations were of only six weeks' duration.

TABLE V  
Sarcoma : type incidence

Type	No.	Percentage of sarcomas
Spindle-cell ... ..	53	25.9
Kaposi ... ..	24	11.8
Round-cell ... ..	21	10.3
Lymphosarcoma ... ..	53	25.9
Mixed cell ... ..	11	5.4
Osteogenic sarcoma ... ..	16	7.8
Rhabdomyosarcoma ... ..	13	6.4
Leiomyosarcoma ... ..	5	2.5
Angiosarcoma ... ..	3	1.5
Gliosarcoma, spongioblastoma ... ..	2	1.0
Gliosarcoma, ependymoma ... ..	1	0.5
Neurofibrosarcoma ... ..	1	0.5
Liposarcoma ... ..	1	0.5
Total ... ..	204	100.0

TABLE VI  
Sarcoma : site incidence

Site	No.	Percentage of sarcomas
Upper limbs ... ..	24	11.8
Lower limbs ... ..	57	27.9
Face, head, mouth ... ..	22	10.8
Eye ... ..	7	3.4
Chest-wall, trunk ... ..	8	3.9
Lymph-nodes ... ..	43	21.0
Uterus ... ..	6	2.9
Male breast ... ..	2	1.0
Bone ... ..	15	7.4
Bladder ... ..	1	0.5
Heart ... ..	1	0.5
Liver ... ..	1	0.5
Ovary ... ..	2	1.0
Abdominal ... ..	10	4.9
Stomach ... ..	2	1.0
Brain ... ..	3	1.5
Total ... ..	204	100.0

*Endothelioma of Spleen.* One tumour of the type described by Ewing (1940) as endothelial sarcoma is included in Table V under 'Lymphosarcoma,' and in Table VI under 'Abdominal.'

*Sarcoma of Stomach.* The two tumours recorded were of the reticulum-cell type of lymphosarcoma.

## SUMMARY AND CONCLUSIONS

One thousand malignant tumours from all parts of Nigeria are analysed as accurately as possible from the available data.

The results are tabulated and commented upon.

The melanomas, liver carcinomas, chronic leg ulcer carcinomas and the Kaposi tumours receive special attention.

An analysis of this type from biopsy and autopsy material cannot give a really accurate picture of the incidence of malignant tumours in Nigeria, but it is felt that it provides information of considerable value at this stage of the country's development.

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We are especially indebted to the late Dr. E. C. Smith, whose collaboration we should have had but for his untimely death by enemy action in July, 1943. To his enthusiasm and unfailing interest we wish to pay this tribute.

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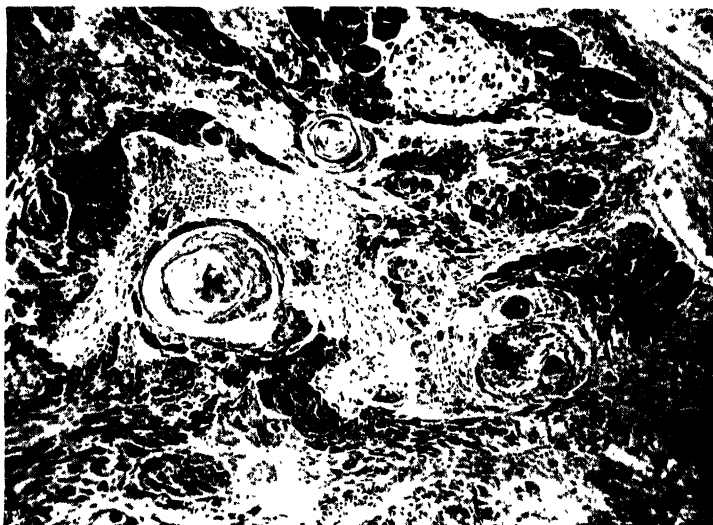


FIG 1. Pigmented acanthoma (melanoma) (  $\times 60$  )

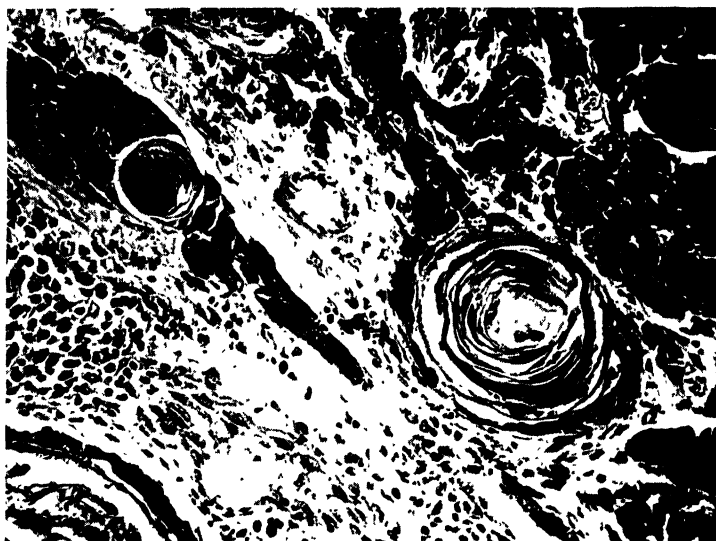


FIG 2. Pigmented acanthoma (melanoma) (  $\times 200$  )





FIG. 1. Squamous carcinoma of the chronic leg ulcer type. ( $\times 11$ .)



FIG. 2. Squamous carcinoma of the chronic leg ulcer type. ( $\times 150$ .)

# EXPERIMENTAL MALARIAL INFECTIONS IN AUSTRALASIAN ANOPHELINES

BY

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AND

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## I. INTRODUCTION

The high rate of malarial casualties in the Australian military forces caused grave concern in the early stages of military operations in the South-West Pacific. In 1943 research work on various antimalarial drugs was initiated by Brigadier N. Hamilton Fairley, then Director of Medicine, Australian Military Forces, and the Land Headquarters Medical Research Unit (A.I.F.) was established at Cairns, North Queensland, under his direction. Soldiers were invited to volunteer as subjects for this research, and were infected with benign or malignant tertian malaria by means of laboratory-bred mosquitoes infected from other soldiers. The plan of the entomological work was prepared by Lieutenant-Colonel I. M. Mackerras, then Director of Entomology, A.M.F., and the present authors were given the task of breeding and infecting anophelines on the very large scale required (Fairley, 1945, 1946*a*, 1946*b*; Fairley *et al.*, 1946).

During the course of the work, over 233,000 engorged mosquitoes were handled, 38,000 dissections were performed, and more than 20,000 infected bites were inflicted. This naturally resulted in the accumulation of a considerable mass of data, the general usefulness of which was lessened by the fact that first consideration was always to maintain the stock of infected mosquitoes at an adequate level, and parasitological or entomological observations could only appear as by-products. For example, we could not afford to sacrifice mosquitoes simply to make comparisons between the infection in different species, nor could we afford laboratory space to run many longevity tests, nor make other purely entomological investigations. However, for each batch of mosquitoes a daily record was made of the number dying and of the results of any dissections performed, which provided the basic information used in this paper.

The behaviour of the parasites in the human host and the early stages of development in the mosquito are discussed elsewhere (Mackerras and Ercole, in the press), while Mackerras and Lemerle (in the press) have described the biology of *Anopheles punctulatus punctulatus* in captivity. In the present paper an attempt is made to summarize our findings in relation to transmission by the mosquito and the conditions which influence it.

## II. MATERIALS AND METHODS

### (a) Availability of Various Species

Cairns was selected for the investigation for several reasons, one of which was the abundance of anophelines. Nine species had been found in the district (Roberts, in the

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press), and one of the most abundant, *A. punctulatus farauti* Lav. (= *moluccensis* Sw. and Sw. de G.), was a proven vector in an epidemic of benign tertian malaria which occurred in Cairns in 1942 (Heydon, unpublished communication). *A. annulipes* Walk. was also abundant, and had been proved earlier to be hospitable to *Plasmodium vivax* and *P. falciparum* (Roberts, 1943).

It was therefore proposed to use bred adults of these two species for the transmission of malaria to volunteers. Several other species, *A. amictus amictus* Edw., *A. amictus hilli* W. and L., *A. bancrofti bancrofti* Giles, and *A. meraukensis* Venh., were collected in small numbers, and incidental observations were made on them.

The local supply gradually diminished, owing to an unusually dry spring and to a vigorous malaria-control programme involving the drainage of hitherto extensive swamps. Supplies were then supplemented by the collection of *A. annulipes* in Brisbane and Townsville, larvae being sent by air to Cairns. But even these areas failed to supply sufficient mosquitoes, and anopheline larvae were then obtained by air from New Guinea. It was found that *A. punctulatus punctulatus* Dön. could be readily collected in pure culture at Milne Bay and that consignments sent by air reached Cairns in a satisfactory condition. However, after about 10 months this source of supply also failed, and we were forced to establish a laboratory colony of *A. punctulatus punctulatus*. This colony flourished for over a year, and a steady supply of adults in excess of actual needs was readily maintained until the work ceased in March, 1946.

Special experiments were set up to test the hospitality to infection of two species not mentioned above, namely, *A. stigmaticus* Sk. from Cairns, and *A. longirostris* Brug from Milne Bay. The full list of species studied is set out in Table I below. Throughout the text, however, for the sake of simplicity the specific, or, in the case of polytypic species, the subspecific, name alone will be used.

#### (b) The Collection of Larvae

Owing to the wide dispersal of anopheline larvae, a team of 12 men, under Lieutenant J. McMahon, was required to collect sufficient for our needs. The collectors waded slowly about the swamps and pools, picking well-grown larvae from the surface by means of small nets, measuring  $1\frac{1}{2}$ –4 in. in diameter, covered with fine mosquito-netting, the whole resembling a minute wire tennis racquet with a long handle. The larvae were transferred to large glass jars containing clean water from the breeding-pool. Overcrowding was avoided, as it increased mortality. When being transported by motor truck, the jars were carried by hand, as the larvae were readily drowned by continuous jolting. Sometimes they were stranded on water-weeds, and were successfully transported adhering to the wet weeds. It was soon learnt that too-frequent visits to a pool led to its failure as a breeding-ground. This was thought to be due not only to the depletion of the adult population, but also to the frequent disturbance by collectors wading through the water, thus rendering it unsuitable for breeding.

When air-transport was employed, about 750–1,000 larvae were placed in water about 2–3 in. deep in a pickle-bottle, the top of which was securely covered by fine netting. Six pickle-bottles were packed tightly in a small wooden crate. Although many larvae died or were devoured by their fellows (for they are cannibalistic when overcrowded and starved), a satisfactory yield was still obtained, provided that the journey did not occupy more than two days. When delays in transport occurred, the small larvae succumbed,

and the adults, which developed from the full-grown larvae, usually fell back into the water and were drowned.

### (c) *Rearing of Larvae*

On reaching the laboratory, the collection was emptied into large enamel basins 12-14 in. in diameter, and predators were removed. These large basins were rather deep, and it was found that larvae preferred shallower water. Small glass soup-plates, 7 in. in diameter and 1½ in. deep, proved satisfactory, and were used consistently for the last two years of the work. At first, water from a natural breeding-pool was used to replenish the breeding-dishes, but tap-water was found satisfactory for well-grown larvae, provided that it had not been heavily chlorinated. When it was necessary to rear larvae from eggs, it was found beneficial to add soil to the breeding-dishes. Various foods were tried, but a cereal food called Farex\* proved very satisfactory and was ultimately used exclusively. Being finely divided and light, it floats for considerable periods --an advantage for surface-feeding larvae like anophelines.

Each morning the pupae were pipetted out into clean water in pint enamel mugs, about 4½ in. in diameter. About 1,000 pupae could be accommodated in each mug. Floats for the emerging adults were unnecessary, provided that the water was clean. Emergence rarely fell below 90 per cent.

### (d) *Emergence of Adults*

The mugs containing the pupae were placed in large cages, consisting of a wooden floor 15 in. by 11 in. and a wooden frame 10 in. high, covered with fine netting and provided with a long sleeve. These 'emergence cages' were covered with wet towels and placed on an ant-proof table. All the mosquitoes had usually emerged in 36 hours, and the mugs were then removed. To reduce overcrowding, the males were taken out by a suction apparatus. Newly emerged females fed readily on carbohydrate food, which was always available to them, as starvation at any age was poorly tolerated.

### (e) *Infection and Maintenance of Adults*

Females were usually kept until they were 48 hours old before being offered a blood-meal, as very few would bite on the first day after emergence. In cool weather it was possible to hold *punctulatus* for several days or even a week, but the Australian species were shorter lived, and the chances of a useful number surviving until the parasites had completed their development became increasingly remote with each passing day.

All food was removed about six hours before feeding, but, as the cages were covered with wet towels, the insects always had access to water. The arm of the gametocyte-carrier was inserted into the cage through the sleeve, and the cage was covered with a blanket. After about 10 or 15 minutes the blanket was removed, and, if many mosquitoes were still unfed, a towel wrung out in hot water was applied to the cage. The moist heat drove the unfed females down on to the arm, and biting and engorgement usually followed.

\* Farex is prepared by Glaxo Laboratories (Aust.) Pty. Ltd. Its composition is stated to be : carbohydrate 75 per cent., protein 14 per cent., fat 3 per cent., minerals (including iron and calcium) 4 per cent., moisture 4 per cent. ; vitamins B<sub>1</sub> and D present.

Sometimes it was necessary to repeat this manoeuvre several times, but it was found useless to persist longer than 45 minutes. In the hottest months—December and January—on the other hand, it was necessary to cool the cages with iced towels for several hours before biting, in order to induce a reasonable proportion of the females to engorge.

Engorged females were transferred to 'standard cages,' consisting of a wooden floor 15 in. by 8 in. and a wire framework 9 in. high, with a covering and sleeve of fine mosquito-netting. Transference was originally made by catching each engorged female in a 3 by 1 in. glass tube, and releasing it in the new cage. However, it was found that they could be collected in the suction apparatus quite satisfactorily by using a fairly wide-bore tubing. If digestion was allowed to proceed over night before transference, no difference in the survival rate was observed between batches treated in this way and others fed at the same time and handled singly in tubes. The number of engorged females was usually limited to 200 per standard cage, giving about 6 cubic in. of space per mosquito.

The cages were kept covered with towels, which were wrung out in water at least once a day. In midsummer, iced water was used. Although the cooling effect was only transient, it helped to offset the adverse effect of the daily routine of picking out the dead mosquitoes and renewing the food.

These cages were stored in a large wooden rack, 6 by 3 by 6 ft., with four tiers of shelves, capable of accommodating 64 standard cages. This rack was built in the centre of the storage-room, and was provided with a shallow overhead tank and hessian curtains which could be lowered or rolled up as required. In hot weather water was allowed to percolate through the hessian curtains and was collected in a large drip-tray provided beneath the rack. Considerable cooling was obtained by playing a fan upon the wet hessian. In cooler weather the water was cut off and the curtains rolled up, and in winter a radiator was used to warm the room.

The most suitable food was found to be fresh apple. Moderately thin slices were laid on the top of the cage and renewed daily. Adults of both sexes fed readily on the freshly cut surface, and would survive well on this food alone. It was necessary to replace the apple daily, as the mosquitoes seemed unable to pierce the cut surface and obtain nourishment from the deeper layers once the surface had dried. Apple was more satisfactory than any other fruit tried; it did not become sticky, and the insects could walk on it without becoming trapped. No special water-containers were necessary, the insects obtaining sufficient liquid either from the apple-juice or from the wet towels. The addition of sugar and water in pads on the floor of the cages was tried, as well as the provision of extra blood-meals after the infecting feed. Neither of these procedures increased survival, and both were abandoned.

When fresh apples were unobtainable, dried sliced apples were substituted, but were only moderately satisfactory. Possibly the juice obtained was less digestible than fresh apple-juice; at all events, the insects frequently became bloated, and would not attempt to take blood unless starved for much longer periods than were necessary when fresh fruit was used. It was, therefore, a particularly unsuitable food for newly emerged females or for those in the infective stage, and we always endeavoured to provide fresh fruit for these mosquitoes. There was no evidence that a diet of apple-juice interfered with the development of the parasites in any way.

Two very important points to ensure satisfactory survival were (i) to maintain a high humidity within the cages, and (ii) to reduce handling and disturbance to a minimum.

(f) *Transmission of Infection to Man*

At the beginning of the investigation, small numbers of infected mosquitoes were transferred to cages 9 by 9 by 9 in. in dimension, the numbers used depending on the sporozoite-rate of the batch and on the number of infections desired. Each volunteer's hand was placed in a cage, which was then covered by a blanket. This method proved slow, tedious and uncertain, and the extra handling was detrimental to the insects. It was found more satisfactory to leave the mosquitoes together in a standard cage, and to allow them to bite the back of the volunteer's hand pressed firmly against the net. The cage was kept partially covered with a moist towel, and the mosquitoes could be attracted to the required area by breathing into the cage. The area of skin exposed depended on the number of infective bites required. We found that the species employed could not pierce ordinary writing-paper, so a paper mask with a hole cut in it was used to confine the mosquitoes to a small area of skin, where they could be watched and counted accurately. Excess females were prevented from biting by blowing them away as they attempted to settle on the skin. If only one or two infections were required, the mosquitoes were handled singly in tubes, and where dissection immediately feeding was complete.

(g) *Dissection Technique*

Small light forceps, with rounded ends meeting accurately at the tip, were used for handling mosquitoes and cover-slips. Straight surgical needles with cutting edges were mounted in metal or wooden handles, and were kept sharp with a fine carborundum stone. When not in use they were stuck into a thick pad of vaselined gauze, to prevent them from rusting. Physiological saline (0.9 per cent. NaCl) was used as dissecting fluid. Mosquitoes for dissection were killed by cyanide. Chloroform was found an unsuitable killing agent, because the gut became fragile and liable to tear during removal, unless dissections were made immediately after death.

The following method of dissection was found most satisfactory :

(i) After identification, the legs and wings were removed as cleanly as possible ; the insect was placed on its side on a 3 by 1 in. slide under the dissecting microscope, and decapitated.

(ii) The neck was placed close to a small drop of saline, and, with the tip of the abdomen pointing away from the dissector, the needle in the left hand was laid flat across the thorax along a line between the first two pairs of legs, and an even, gentle, downward pressure was exerted. This usually resulted in the salivary glands being extruded through the neck. By gentle dissection the glands could be then freed. To prevent them from separating when the cover-slip was applied, we found it best to draw the glands together and leave them stranded just at the edge of the saline. The body was then removed, any pieces of detached chitin cleaned away, and a cover-slip placed over the glands.

(iii) To dissect the mid-gut, the body of the insect was placed on a *dry* slide, and the terminal segments of the abdomen were nicked in the usual way. The tip of the abdomen was then held by a needle, and the rest of the body was drawn slowly and carefully away. The Malpighian tubules were stripped back by this manoeuvre, and the gut was left cleanly extended. A drop of saline was added quickly, and a cover-slip was then applied.

The unstained preparations lying in physiological saline were examined for sporozoites or oöcysts with a 4 mm. objective and 10× ocular. Occasionally the identification of very small or doubtful cysts necessitated examination with an oil-immersion objective.

The intact salivary-gland preparations were examined with the 4 mm. objective, the glands were then crushed, by pressing on the cover-slip with the point of a needle, and the preparation was examined again. If glands are crushed before they are examined, a very light infection may be missed, as scanty sporozoites may become widely dispersed. A practised dissector can dissect and examine the salivary glands and mid-gut of upwards of 20 mosquitoes in an hour, but 15 per hour represents a good average if many negatives are encountered.

The dissection of dead mosquitoes was not usually satisfactory, but it was surprising how much of the glands and mid-gut, particularly the former, could be extracted from apparently rotten specimens. Sporozoites and large oöcysts might appear normal, even though decomposition of the tissues in which they lay was well advanced. Even when no traces of the glands could be found, sporozoites might at times be picked up by careful examination of the anterior thoracic tissues. Young oöcysts were very difficult to find in even partly decomposed guts, and no reliance could be placed on a negative result unless the gut was absolutely fresh. Incidentally, it became evident, as the work progressed and more dissectors were trained, that much more reliance may be placed on the results of examination of salivary glands than of mid-guts; in other words, an unpractised dissector is more likely to miss small oöcysts than sporozoites.

### III. BEHAVIOUR OF ANOPHELINES IN CAPTIVITY

Two characteristics which are particularly important in the transmission of malaria by any anopheline, whether in the field or in the laboratory, are (i) its avidity for human blood and (ii) its longevity.

#### 1. AVIDITY FOR HUMAN BLOOD

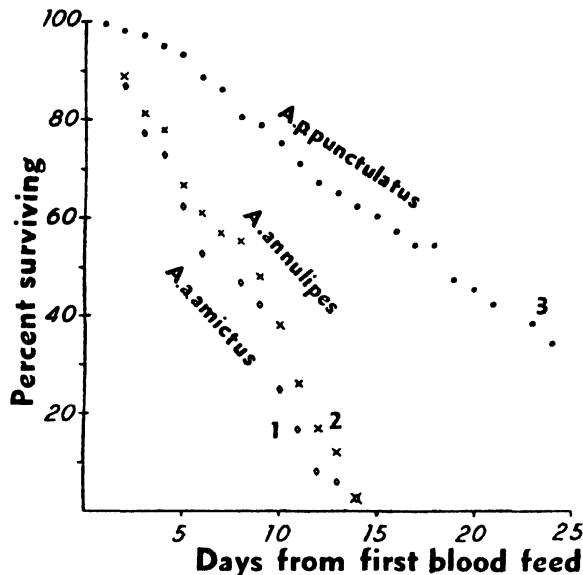
All the anophelines studied by us were found to take human blood, but their readiness to bite in captivity varied considerably. *Punctulatus* fed most readily, and a high percentage would bite freely without stimulation at any hour of the day or night, and in all weathers, except in extremely hot weather. We have on several occasions engorged 1,200 females at a single 45-minute biting-session. *Farauti* occasionally bit very freely, but was more capricious in its behaviour than its relative, and generally had to be encouraged with hot moist towels. In earlier experiments (Roberts, 1943), only 34 per cent. of 535 females used engorged on gametocyte-carriers. However, some mosquitoes in use during one autumn were noted to be extremely vicious biters, and a swarm of females would descend on the gloved hand of the attendant each morning, while he was removing dead mosquitoes from the cage.

*Annulipes* and *amictus* were fed in moderate numbers, but were at all times very fickle biters in captivity, although one of us (Roberts, 1943) had previously not had so much trouble with *annulipes*, 49 per cent. of 535 females engorging on the gametocyte-carriers, as compared with 34 per cent. of *farauti*. Very small numbers of *bancrofti* and *hilli* were used, and the proportion engorging was not recorded; they did not appear to bite very readily in captivity.

*Longirostris* is known to attack man only on rare occasions in the field, and proved a very poor biter in captivity. *Stigmaticus* has not been recorded as biting man in nature, and also bit very reluctantly in the laboratory. Much time and patience were needed to induce even a few of these two anophelines to engorge on gametocyte-carriers.

## 2. LONGEVITY IN CAPTIVITY

Certain factors, which obviously have a profound effect on the survival of mosquitoes, were kept as nearly at an optimum as possible. These were protection from enemies and pathogens, shelter from sun and wind, maintenance of high atmospheric humidity, and a supply of fresh food. The cages were kept in shaded positions, out of draughts, and protected from ants. They were wrapped in wet towels, and fresh carbohydrate food was supplied daily. Dead mosquitoes were removed daily, and the floor of the cage was kept dry. As each batch of mosquitoes died or was destroyed, the cage was scrubbed, immersed in boiling water for several minutes, rinsed thoroughly, and left in the sun to dry. The towels were boiled once weekly. Other factors, which varied from time to time, included species of anopheline used, temperature, degree of crowding, amount of handling of fed adults, and larval environment.

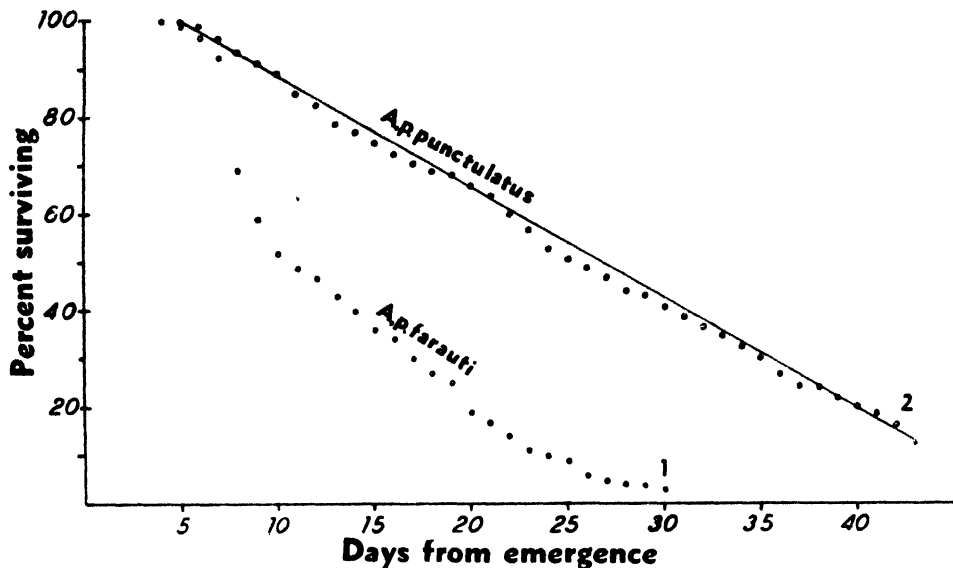


GRAPH 1. Survival curves, October, 1943. Mean temperature 79° F. 1.—*A. amictus amictus*: curve based on 107 mosquitoes. 2.—*A. annulipes*: curve based on 128 mosquitoes. 3.—*A. punctulatus punctulatus*: curve based on 362 mosquitoes.

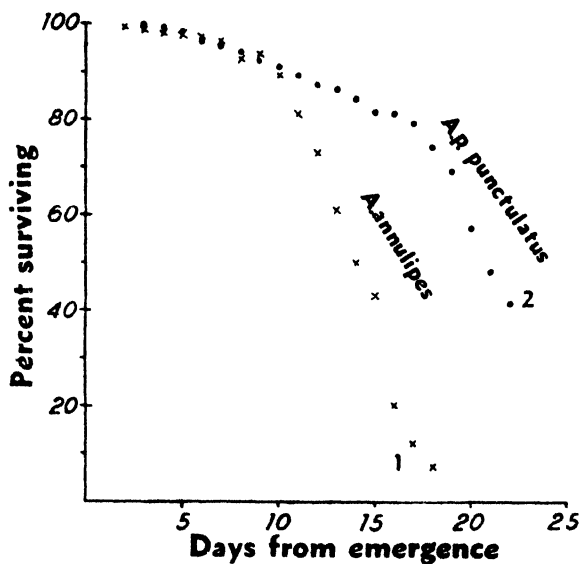
## (a) Differences between Species

It is obvious that, since wide variation may occur in the same species under different conditions, even at the same time of year (see graphs 6–9), great caution is necessary in drawing deductions from survival curves for different species. Tests designed to give all species under comparison the same chance of survival would need to be repeated many times with consistent results before any dogmatic statement could be made. We have not large numbers for comparison, nor many replications, but we consistently found that *punctulatus* survived better in captivity than any other species. This was first appreciated in the spring of 1943, when the observations shown in graph 1 were made. It will be seen that, at the end of the second week after a blood-meal, and when the mosquitoes





GRAPH 2. Survival curves, May, 1944. Mean temperature 73° F. 1.—*A. punctulatus farauti*: curve based on 241 mosquitoes. 2.—*A. punctulatus punctulatus*: curve based on 601 mosquitoes; straight line represents a daily loss of 2.3 per cent. of original population between the 5th and the 43rd day.

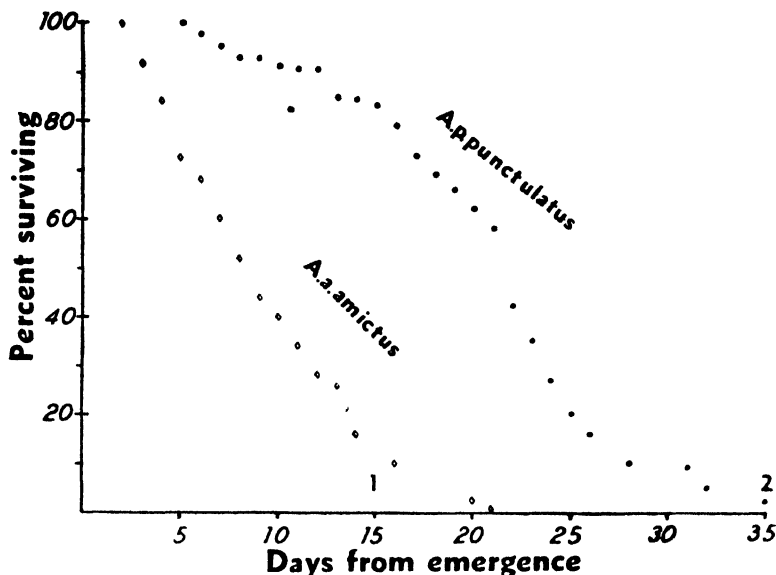


GRAPH 3. Survival curves, November, 1943. Mean temperature 79° F. 1.—*A. annulipes*: curve based on 353 mosquitoes. 2.—*A. punctulatus punctulatus*: curve based on 417 mosquitoes.

were reaching the infective stage, the local species were practically all dead, whereas over 60 per cent. of the original *punctulatus* population remained.

The superiority of *punctulatus* is further illustrated in graphs 2-4, in which this race is compared in parallel series with *farauti*, *annulipes* and *amictus* respectively. Indeed, the maximum survival of any other species, under any of the laboratory conditions prevailing, rarely equalled the minimum summer survival of *punctulatus* shown in graph 6.

As regards the anophelines which occurred naturally at Cairns, little difference was seen between the three common species (graph 5). The mosquitoes in the batches illustrated were given a single blood-meal, and were not overcrowded. The climatic conditions were favourable (early spring). Very little difference in survival was evident until after the 5th day; thereafter the percentage for *amictus* fell consistently below the others, which



GRAPH 4. Survival curves, January, 1945. Mean temperature 76.5° F. 1.—*A. amictus amictus*: curve based on 50 mosquitoes. 2.—*A. punctulatus punctulatus*: curve based on 136 mosquitoes.

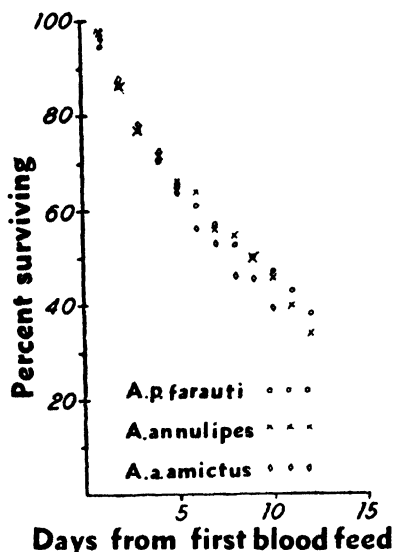
appeared to be surviving about equally. These batches were not infected, and they were destroyed on the 10th or 12th day, so that the ultimate form of the curve was not determined.

*Hilli* was only compared with *farauti* in a series of captured wild females; they survived poorly. The numbers of the remaining species handled, namely, *meraukensis*, *bancrofti*, *stigmaticus* and *longirostris*, were too few to permit any conclusions on their capacity to survive in captivity, though a few individuals of the last three lived long enough to show sporozoites in the glands.

#### (b) Variations in Environment

These factors will be considered only in relation to *punctulatus*, which was in continuous use for over two years. Similar tendencies were noted in other species, but they were only used irregularly, and our records of them are therefore incomplete.

In *punctulatus*, the death-rate, i.e., the number dying each day expressed as a percentage of the survivors on the preceding day, was usually low (about 1-2 per cent.) during the first 10 days of life, and seldom exceeded 3 per cent. The rate then usually increased rather suddenly, and remained high for several days. Thereafter it fluctuated considerably, but tended to increase slowly, until a sharp rise to 100 per cent. occurred on the day when the last mosquitoes died. The critical point in the life of a mosquito population, when the first sudden increase in death-rate occurred, together with the magnitude of the increase, determined the form of the survival curve. Usually it was sigmoid, but sometimes (e.g., graph 2) it approximated to a straight line, when the death-rate remained uniformly low until the last week of life. Any factor causing abnormal activity hastened the appearance of the critical point. These factors included rise of temperature and disturbance, whether due to overcrowding or to handling.



GRAPH 5. Survival curves, September, 1943. Mean temperature 75° F. 1.—*A. punctulatus farauti*: based on 684 mosquitoes. 2.—*A. annulipes*: based on 993 mosquitoes. 3.—*A. amictus amictus*: based on 187 mosquitoes.

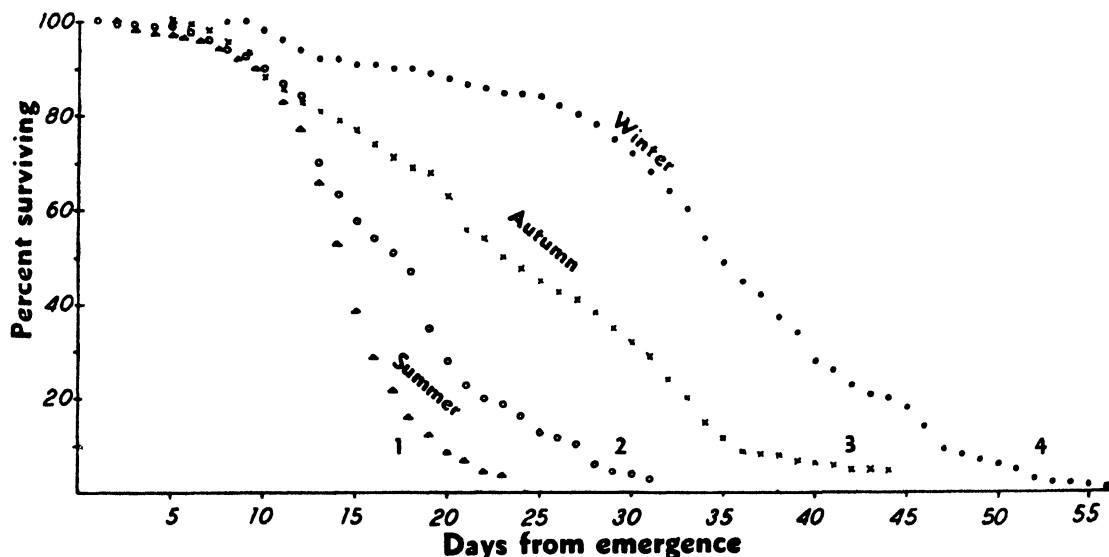
(i) *Season*. There was definite correlation between season and survival rate, mosquitoes dying more rapidly as the daily temperatures increased. This may be seen in graph 6, in which survival curves for different seasons of the year are shown. During midsummer the first death-rate exceeding 10 per cent. usually occurred about the end of the second week (on the 13th day in curves 1 and 2); in spring and autumn it was delayed for another week or so (21st day, curve 3); and in midwinter it was still further delayed (34th day, curve 4).

Climatic conditions were least favourable to survival during the period embraced by curve 1. The mean daily temperatures varied from 78° to 85° F., and only rarely fell

below 80° F. In curve 2 the daily means varied from 75° to 85° F., but only exceeded 80° during the first nine days. In the period embraced by curve 3 the weather had become milder (means 72–77° F.), and during the fourth week it became relatively cold, the average mean for the week being 68° F. In the fifth week the mosquitoes were moved to a new laboratory, in which temperature-control was sufficient to prevent the mean falling below 70° F. In the period embraced by curve 4 the mosquitoes remained throughout their existence in a mild, equable climate, the daily means varying from 70° to 75° F.

The mean temperatures (in degrees Fahrenheit) computed for weekly periods for the four curves were :

- Curve 1. 80, 81, 83, 82.
- Curve 2. 84, 79, 77, 77, 77, 78.
- Curve 3. 76, 75, 74, 68, 73, 75, 72.
- Curve 4. 72, 73, 74, 72, 73, 71, 73, 73.

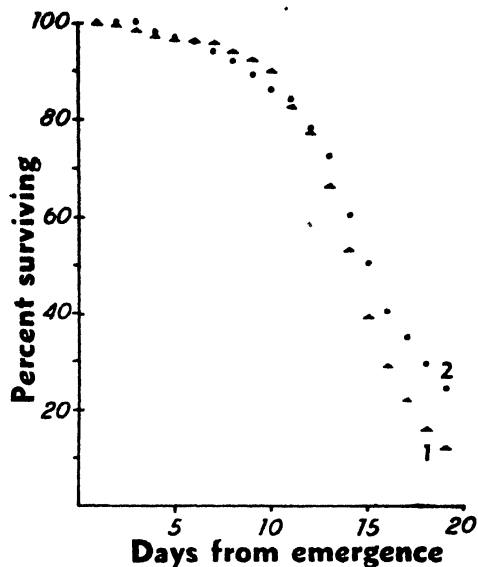


GRAPH 6. *A. punctulatus punctulatus*: influence of season on survival. 1.—Summer: curve based on 1,119 mosquitoes, emerged Nov. 27th–Dec. 2nd, 1943. 2.—Summer: curve based on 629 mosquitoes, emerged Jan. 13th, 1944. 3.—Autumn: curve based on 1,194 mosquitoes, emerged April 16th, 1944. 4.—Winter: curve based on 789 mosquitoes, emerged July 22nd, 1944.

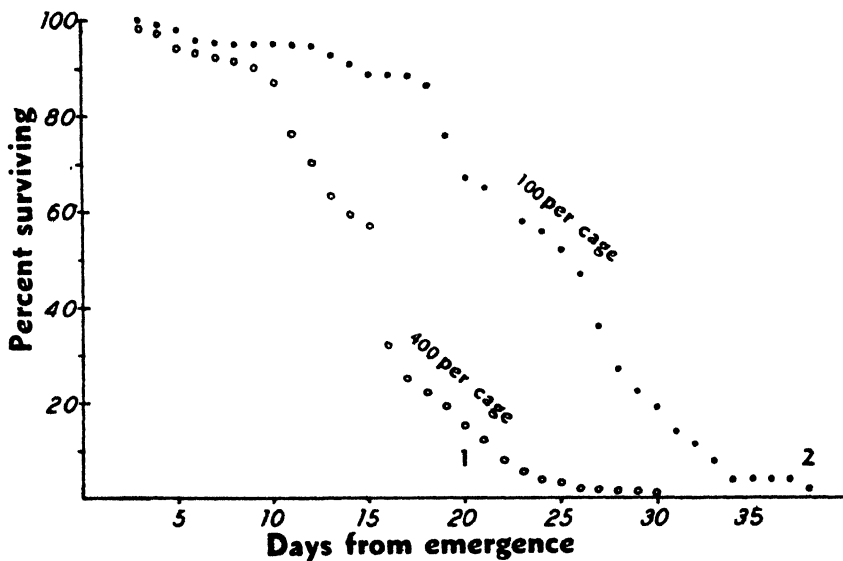
It is interesting to note that a survival curve for bred females, fed once on blood and kept under natural climatic conditions at Salamaua, New Guinea, was essentially similar to the summer curve for laboratory stocks at Cairns (graph 7).

(ii) *Overcrowding*. Overcrowding adversely affected survival, and it was noticeable that a degree of overcrowding tolerable in winter had disastrous effects in midsummer. The effect of overcrowding in hot weather may be seen in graph 8.

(iii) *Handling*. Frequent handling of the cages, and transference of aged mosquitoes from one cage to another, no matter how carefully done, had the same effect as over-



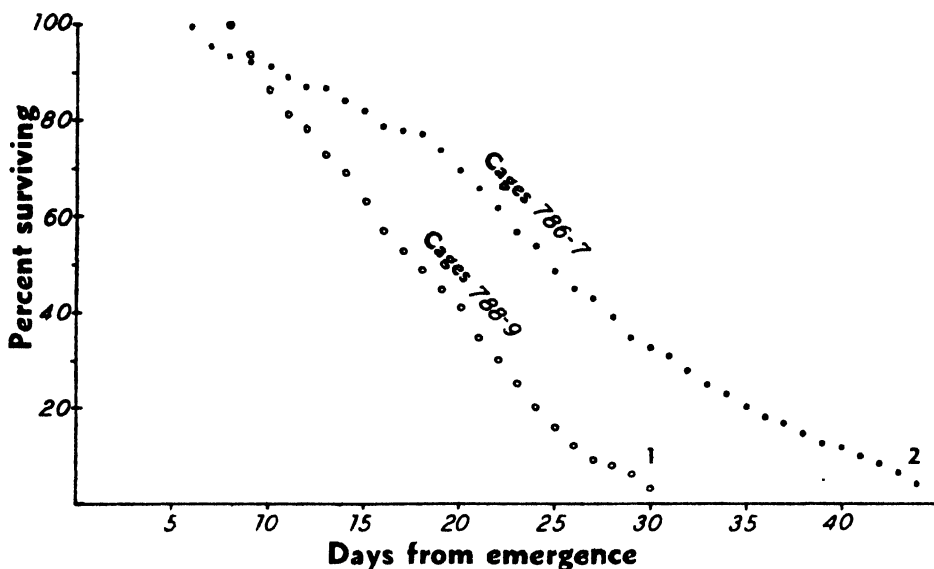
GRAPH 7. *A. punctulatus punctulatus*: survival of bred mosquitoes. 1.—At Cairns (from graph 6). 2.—At Salamaua, New Guinea: curve based on 260 mosquitoes.



GRAPH 8. *A. punctulatus punctulatus*: influence on survival of overcrowding in hot weather, Feb., 1944. Mean temperature 77° F. 1.—400 mosquitoes per cage (3 cubic in. per insect). 2.—100 mosquitoes per cage (12 cubic in. per insect).

crowding, i.e., it shortened life by keeping the insects in a state of abnormal activity. The very best survival rates in hot weather were obtained in cages which had the minimum handling, i.e., they were not removed from the rack, and no attempt was made to pick out the dead mosquitoes. In these cages the mosquitoes were only subject to the disturbance caused by replenishing food and water. In one trial five cages were set up, each containing 100 mosquitoes; four were subject to the routine daily handling, and one was untouched. At the end of four weeks the former contained from 27 to 33 live mosquitoes each, while 60 were alive in the untouched cage.

(iv) *Other Adverse Factors.* The influence of larval environment was not studied particularly, but it is well known that it is important in certain other insects, e.g., house-



GRAPH 9.—*A. punctulatus punctulatus*: variation in survival, due to unknown causes, occurring in consecutive batches of mosquitoes fed on the same donor. Mean temperature 76° F. 1.—Batches 788 and 789: curve based on 349 mosquitoes, emerged March 10th, 1945. 2.—Batches 786 and 787: curve based on 347 mosquitoes, emerged March 9th, 1945.

flies. We tried to give the larvae optimum conditions throughout, but, when dependent on outside sources, some were undoubtedly starved in transit. Certain anomalous survival curves may have been due to differences of this kind. At one period, too, abnormal death-rates coincided with hammering during structural alterations in the laboratory, though why the vibrations should have affected some batches and not others was not clear.

An example of the extreme variation which may occur between consecutive batches of the same species, fed on the same donor and maintained side by side under apparently identical conditions, may be seen in graph 9, which indicates very well the caution which is necessary in making any comparisons of survival rates.

## IV. SUSCEPTIBILITY TO INFECTION

## 1. SPECIES INFECTED

Nine Australasian anophelines, seven from the mainland of Australia and two from New Guinea, were experimentally infected. The species tested and the species of *Plasmodium* are set out in Table I. The majority of infections were observed to the sporozoite stage, but a few, because of paucity of numbers and failure of mosquitoes to survive long enough, were only recorded in the oöcyst stage. It is noteworthy that all species tested became infected, only *longirostris* and *stigmaticus* showing any indication of relative insusceptibility.

Details of the number of infected mosquitoes of each species observed and the degree of infection recorded in them are set out in Tables II and III

TABLE I  
Anophelines experimentally infected with malaria

Mosquito	Source	Plasmodium
Subgenus <i>Myzomyia</i>		
<i>A. punctulatus punctulatus</i> Donitz	New Guinea	<i>P. vivax</i> , <i>P. falciparum</i> <i>P. malariae</i>
<i>fausti</i> Laveran	Queensland	" "
<i>A. annulipes</i> Walker	"	" "
<i>A. amictus amictus</i> Edwards	"	" "
<i>hull</i> Woodhill and Lee	"	<i>P. falciparum</i> (oöcysts only)
<i>A. meraukens</i> Venhuis	"	<i>P. vivax</i> (oöcysts only)
<i>A. longirostris</i> Brug	New Guinea	" ( " ), <i>P. falciparum</i>
Subgenus <i>Anopheles</i>		
<i>A. bancrofti bancrofti</i> Giles	Queensland	<i>P. vivax</i> , <i>P. falciparum</i>
<i>A. stigmaticus</i> Skuse	"	" "

## 2. RELATIVE SUSCEPTIBILITY

It is well recognized that differences in susceptibility to malarial infections exist among anopheline species. Boyd and Earle (1939) found that the Mexican *A. pseudopunctipennis* was distinctly inferior to *A. quadrimaculatus* in its susceptibility to strains of *P. falciparum* from Mexico and Florida. In Panama, Rozeboom (1938) found that *A. albitarsis* was less susceptible to the indigenous strains of *P. falciparum* than *A. albimanus* (the important vector of the region). More surprising results were obtained by Boyd, Carr and Rozeboom (1938) in their studies of the relative susceptibility of *A. albimanus* and *A. quadrimaculatus*. They found that the Neotropical species, *A. albimanus* from Cuba, was highly susceptible to *P. vivax* and *P. falciparum* from its own region, but distinctly refractory to strains of these parasites from Florida, whereas *A. quadrimaculatus* was equally susceptible to strains from both regions. Shute (1940) reported repeated failures to infect English anophelines, *A. maculipennis* var. *atroparvus*, with African and Indian strains of *P. falciparum*, although these mosquitoes were highly susceptible to strains of the same parasite from Italy, Sardinia and Roumania, and also to *P. vivax* from tropical as well as from temperate regions.

For a considerable time workers have realized that great variations in the infectivity of gametocytes occur, and that infection-experiments are worthless unless adequately

controlled. In our own experience, a qualitative change from highly infectious to non-infectious was observed in as short a time as eight hours in *P. vivax*, without any reduction in the total count, and a very marked decrease in infectivity in 24 hours (possibly less) in *P. falciparum*. The existence of persistent non-infectors among *P. falciparum* gametocyte-carriers also complicates the problem (Mackerras and Ercole, in the press). Russell and Mohan (1939) stressed the importance of using a known susceptible species as a control, employing insectary-bred *A. stephensi* for this purpose in their study of Indian anophelines. The American workers used the known vectors, *A. quadrimaculatus* in Florida and *A. albimanus* in Panama, as controls. Shute, however, was not able to control

TABLE II  
*P. vivax*: number of dissections and types of infection recorded

Mosquito	No. dissected	No. positive	Heaviness of infection	
			Mid-gut	Salivary glands
<i>punctulatus</i> ...	10,673	7,281	All grades	All grades
<i>farauti</i> ...	1,003	498	" "	" "
<i>annulipes</i> ...	1,399	650	" "	" "
<i>amictus</i> ...	122	59	" "	" "
<i>meraukens</i> ...	1	1	Light	
<i>bancrofti</i> ...	30	12	All grades	All grades
<i>longirostris</i> ...	11	5	" "	Nil in 5
<i>stigmaticus</i> ...	8	6	Light	Light

TABLE III  
*P. falciparum*: number of dissections and types of infection recorded

Mosquito	No. dissected	No. positive	Heaviness of infection	
			Mid-gut	Salivary glands
<i>punctulatus</i> ...	20,405	14,571	All grades	All grades
<i>farauti</i> ...	969	352	" "	" "
<i>annulipes</i> ...	1,184	453	" "	" "
<i>amictus</i> ...	206	84	" "	" "
<i>hilli</i> ...	20	3	Light	
<i>bancrofti</i> ...	25	1		Light
<i>longirostris</i> ...	5	1		"
<i>stigmaticus</i> ...	7	6	All grades	Heavy

his experiments by using a tropical species simultaneously, and the question therefore arises whether any of his gametocyte-carriers were infectious. The fact that exflagellation was frequently observed, and the formation of ookinetes confirmed in one carrier, makes it reasonable to assume that the parasites were not wholly to blame.

Roberts (1943) made a comparative study of two Australian species, *farauti* and *annulipes*. Mosquitoes were fed simultaneously and housed under identical conditions until dissection. No difference in susceptibility to *P. vivax* and *P. falciparum* was found in these species. We have confirmed these observations, and made some comparisons between other species.



We have attempted to assess the relative susceptibility of species by comparing our records of (a) the intensity of infection, (b) the time required for sporozoites to appear, and (c) the incidence of degenerative changes in the oöcysts. The ideal time to compare the intensity of gut infection is just prior to sporulation, when the oöcysts are readily seen but before they have begun to burst and disappear. For comparison of gland infections, the dissection should be performed when the infection is maximal, i.e., within the first week or 10 days after invasion of the glands, since we usually found that the heaviness of infection diminished steadily thereafter.

Comparison of the time required for sporozoites to develop requires a fairly large number of dissections before and after sporulation. We seldom had sufficient of the rarer mosquitoes to permit many dissections, and our results with these species are therefore incomplete in many details.

With regard to the incidence of degeneration in the oöcysts, it is evident that this may be due to unequal viability of the parasite, as well as to variable resistance of the host. The following observations made on a highly susceptible host—*punctulatus*—illustrate the importance of the former factor. In *P. falciparum* infections it was usual to find that the infectivity of the gametocytes was low at the beginning and again at the end of the gametocyte wave, and that it was at a maximum at the crest of the wave. If mosquitoes were fed early in the wave they usually failed to become infected, and, even if infections were set up, many oöcysts might die. For example, mosquitoes fed on the first day (gametocyte count 620 per c.mm.) showed 100 per cent. oöcyst-rate, but a sporozoite-rate of only 42 per cent. The maximum number of oöcysts seen was 10 per gut, and in many mosquitoes all of them became chitinized. A few days later, at the crest of the gametocyte wave (count 2,400 per c.mm.), another batch of mosquitoes was fed, and in it both rates were 100 per cent., numerous oöcysts being present. Some of these died, but their death had no effect on the sporozoite-rate, since sufficient normal oöcysts were present in every mosquito to ensure that the salivary glands became infected.

Further evidence that death of varying proportions of the oöcysts was a reflection of the varying viability of the parasite was obtained by a study of the infection in mosquitoes fed at intervals after a single dose of a gametocidal drug. For example, mosquitoes were fed at six-hourly intervals after a dose of 10 mgm. plasmoquine had been administered to a patient with 680 *P. vivax* gametocytes per c.mm. At six hours the average number of oöcysts formed was 160 per gut, range 40–300, and only about 3 per cent. failed to complete their development. The number of oöcysts formed diminished steadily, and after 24 hours (gametocytes 720 per c.mm.) the average per gut was only 14, range 2–42, whilst the percentage dying had risen to 43. When a similar dose was given to a patient with *P. falciparum* gametocytes, the effect was more rapid, the number of oöcysts diminishing and the sporozoite-rate falling to zero in 15 hours.

There is no doubt, then, that many immature or senile parasites, or those damaged by drugs, may fail to complete their development in a highly susceptible host, and that the mere occurrence of dead oöcysts is not good evidence of insusceptibility. When, however, there is a marked difference in their incidence in comparable series, this may indicate some inimical conditions in the gut or body cavity of those insects which appear inhospitable.

Our observations on relative susceptibility may be most conveniently presented under the species studied.

*A. punctulatus farauti*, *A. annulipes* and *A. amictus amictus*

At the beginning of the work, we lacked means to discriminate between good and bad gametocyte-carriers, and we therefore fed our mosquitoes, which were mixed cultures of the above three species, on all patients with gametocytes in their blood. We chose the highest count available for the initial feed for each batch, and subsequently offered the mosquitoes feeds on at least two other carriers. As it is not known what proportion of those coming to dissection had actually fed on the later donors, the figures given in Table IV are not strictly comparable. However, we have included them here because they are the most extensive series that we have, and because they show very little difference in infection-rates, and none that could be regarded as significant. The range of oöcysts in each species was from one to numerous, and the salivary-gland infections varied from light to heavy.

TABLE IV  
Infection-rates in three species after several blood-meals

Parasite ... ..		<i>P. vivax</i>		<i>P. falciparum</i>	
Donors' gametocytes per c.mm. ...		Varied from 10 to 2,000		Varied from 10 to 7,700	
	Species	No. dissected	Percentage positive	No. dissected	Percentage positive
Gut infections	<i>farauti</i>	174	65	182	47
	<i>annulipes</i>	289	61	421	48
	<i>amictus</i>	36	66	156	45
Gland infections	<i>farauti</i>	268	50	400	38
	<i>annulipes</i>	476	58	310	40
	<i>amictus</i>	40	45	29	48

The sporozoite-rate for *amictus* infected with *P. falciparum* is certainly erroneous, the error being probably due to the small sample examined. Sporozoite-rates usually fell below oöcyst-rates and obviously can never exceed them.

Small batches of mosquitoes were sometimes fed on one donor and dissected while the infection was still in the oöcyst stage. We have observed 100 per cent. infection-rates in both *farauti* and *annulipes* in these batches. *Amictus* was never abundant enough to make adequate comparisons, but in the few that were dissected the infections appeared similar to those in the other two species.

The time required for the development of sporozoites was approximately the same in these three species. In August *P. vivax* required 14 days in *farauti* and *annulipes*; *amictus* was scanty in these batches, but development appeared to be occurring at the same rate. In October *farauti* was less numerous, and the time for development was 10 days in *annulipes* and *amictus* (insufficient data for *farauti*). The times for *P. falciparum* sporozoites to appear also seemed independent of the species. In August they were recorded in the glands on the 17th day in *annulipes* and on the 18th day in *farauti*, in October on the 13th day in *amictus* and on the 14th in *annulipes*.

Degenerating oöcysts were sometimes seen in all three species, but they were not noted as more abundant in one than in another.

*A. punctulatus punctulatus*

When we began to use this species, regular collection on the mainland ceased, and we therefore never had an abundant supply of all species at the same time, and seldom fed mixed batches on gametocyte-carriers.

In Table V the findings in a mixed batch of *punctulatus*, *annulipes* and *amictus* are set out, and it will be seen that there was no obvious difference between them. Development to sporozoites occurred equally rapidly in all, sporozoite-rates were similar, and there was no difference in the incidence of degeneration in the oöcysts.

TABLE V  
Infection-rates with *P. vivax* and range of oöcysts per gut in three species after one blood-meal

Donors' gametocytes per c.mm		520	
Species	No dissected	Percentage positive	Oöcysts per gut
<i>punctulatus</i>	31	78	1-30
<i>annulipes</i>	25	80	8-40
<i>amictus</i>	20	80	1-50

Similar results were obtained when *punctulatus* and *farauti*, or *punctulatus* and *annulipes*, were fed on *P. falciparum* gametocyte-carriers. The result of one of these tests is shown in Table VI.

Development to sporozoites in the batch cited in Table V occurred on the 10th day in all three species. With *P. falciparum* in the same month (October) first positive glands were recorded on the 13th day in *punctulatus* and *amictus*. In March they were recorded on the 14th day in *farauti* and *punctulatus*. Thus the speed of development of both parasites varied with the temperature and not with the insect host. The relation of season to the rate of development was carefully studied for *punctulatus*, and the developmental periods and corresponding temperatures at monthly intervals are given elsewhere (Mackerras and Ercole, in the press).

*A. bancrofti*

A moderate number appeared from time to time in the local collections, and sufficient were fed on *P. vivax* carriers to show that heavy infections, comparable in every way with those in *farauti* and *annulipes*, could develop in both mid-gut and salivary glands. Very few were fed on *P. falciparum* carriers, who unfortunately proved to be poor in parallel feedings in the above-mentioned species, and only one light salivary-gland infection was recorded in *bancrofti*.

*A. meraukensis*

A single infected mosquito was found in a batch of local mosquitoes fed on several *P. vivax* gametocyte-carriers. The mid-gut infection was a light one, comparable with those seen in *farauti*, *annulipes* and *amictus* fed at the same time.

*A. amictus hilli*

Wild females were captured one evening near their breeding-place and were fed on gametocyte-carriers, along with large numbers of *farauti* captured at the same time, as well as bred *punctulatus*. Only one successful experiment was performed. *P. falciparum* gametocytes numbered approximately 800 per c.mm., but were not highly infectious, as the controls were only lightly infected. The results are set out in Table VI.

TABLE VI  
Infection-rates with *P. falciparum* and range of oöcysts per gut in three species  
after one blood-meal

Donors' gametocytes per c.mm. . . . . 800			
Species	No. dissected	Percentage positive	Oöcysts per gut
<i>punctulatus</i> ...	25	72	1-10
<i>farauti</i> . . .	31	77	1- 8
<i>hilli</i> ... . .	10	30	1- 3

By the time that the infection was established in the salivary glands of both subspecies of *A. punctulatus*, there were no *hilli* alive in this particular batch; thus the infection was not followed through to its final stage. It is possible that *hilli* is not as hospitable to infection with *P. falciparum* as the controls, but the numbers were so small and the gametocyte-carrier was so poor that it would not be justifiable to assume that there is any real difference. Moreover, during the epidemic of benign tertian malaria at Cairns in 1942, one infected specimen out of 79 examined was found with sporozoites in the salivary glands.

*A. stigmaticus*

This small delicate anopheline is rare in the Cairns district, where it was found breeding only in clear rock-pools in the foot-hills of the Great Dividing Range. A number of adults, reared from larvae obtained from the above source, were given the opportunity to engorge on selected *P. vivax* and *P. falciparum* donors. Engorgement of a few females was obtained only after the expenditure of much effort and patience.

Of eight *stigmaticus* which fed on a *P. vivax* donor, six became infected. Gland infections occurred in two of the three females which survived for 12 days. The infection-rate in a control batch of *punctulatus* was 95 per cent. Neither gland nor gut infections in *stigmaticus* were as heavy as in *punctulatus*, and, furthermore, the former had relatively large numbers of degenerate oöcysts.

Of seven *stigmaticus* fed on a *P. falciparum* donor giving an infection-rate of 99 per cent. in control *punctulatus*, six became infected. Two females survived to the 14th day, when one showed a heavy gland infection. Gut infections compared very favourably with the control species, but here again *stigmaticus* had a good number of degenerate oöcysts.

*A. longirostris*

Adults of this species were reared from larvae forwarded from Milne Bay, New Guinea. In the first experiment a few females were fed on a *P. vivax* gametocyte-carrier,

and two of them survived to the 14th day, when one was found to have five small to medium-sized cysts on the gut, the glands being negative. Two *farauti* and three *punctulatus*, which had access to the same donor at the same time, were also dissected; one of the former and two of the latter were positive, having lightly infected glands. The gametocyte-carrier was, unfortunately, a poor one, and little can be deduced about the hospitality of *longirostris* to *P. vivax*, except that the growth of the oöcysts was definitely retarded. In another experiment nine *longirostris*, which had fed on a good donor (gametocytes 1,360 per c.mm.), were dissected on the 21st day, by which time the infection was well established in the glands of a control batch of *punctulatus*. All the nine *longirostris* had negative glands, but four showed evidence of infection, having from two to numerous large dead cysts on the gut wall. The salivary-gland infection in *punctulatus* was 100 per cent. and very heavy. We were not able to repeat the experiment, but the results obtained indicate that *A. longirostris* is relatively inhospitable to infection with *P. vivax*.

A few *longirostris* were fed on a *P. falciparum* gametocyte-carrier (gametocyte count 800 per c.mm.). After 22 days five were dissected, one of which had a very light infection of the glands, the gut being negative. All the others were completely negative. One specimen out of four *punctulatus* fed at the same time had heavily infected glands and 15 sporulating cysts were still present on the gut wall. However, this gametocyte-carrier was not a very good one, and subsequent dissections of the *punctulatus* batch gave many negatives, the sporozoite-rate being only 33 per cent. We can only say from this experiment that *P. falciparum* may complete its development in *longirostris*, but we have insufficient data to compare its susceptibility with that of *punctulatus*. This finding suggests that some *P. vivax* might have completed their development if we had tried often enough.

To sum up, then, all the Australasian species studied became infected to some extent. No difference in susceptibility to *P. vivax* was found in *punctulatus*, *farauti*, *annulipes*, *amicus* or *bancrofti*; *stigmaticus* proved slightly inferior, and *longirostris* decidedly inferior to *punctulatus*. No difference in susceptibility to *P. falciparum* infection was observed in *punctulatus*, *farauti*, *annulipes* or *amicus*, but *stigmaticus* proved slightly inferior to *punctulatus*. The numbers dissected were inadequate to permit other comparisons.

Our work was carried out exclusively with *P. vivax* strains from New Guinea and with *P. falciparum* from New Guinea and the Solomon Islands. We did, however, have one opportunity to feed mosquitoes on a *P. vivax* gametocyte-carrier who had acquired his infection in Syria. We fed mosquitoes on four successive days, and, although the numbers of gametocytes were adequate and we found exflagellating males on each occasion, the result was an almost entire absence of infection in the mosquitoes. We found one lightly infected *annulipes* (two oöcysts) out of 80 dissected, but no infection was detected in 62 *farauti* or 15 *amicus*. We were, for obvious reasons, unable to control this experiment, and therefore cannot be certain whether the gametocytes were non-infectious or whether the Australian anophelines were refractory to the particular strain of parasite.

### 3. INDIVIDUAL VARIATIONS IN SUSCEPTIBILITY

Individual, as well as specific, variation in susceptibility to *Plasmodium* infection is a well-recognized phenomenon among mosquitoes, and has been studied by a number of workers, notably by Huff (1927, 1929, 1931, 1934, 1935, 1940), James (1931), and Boyd and Russell (1943). Using avian malaria, Huff found that *C. pipiens*, when given two

infective feeds, either developed two broods of parasites or failed to become infected at all, and he was able by selection to establish relatively susceptible and relatively insusceptible strains of this mosquito. James reported a wide range of individual susceptibility, citing oöcyst counts varying from 5 to 365 in *A. maculipennis* given the same opportunity to become infected. He suggested that there might be differences in the permeability of the lining membrane of the mid-gut. He found, however, that, in contrast to Huff's experience with *C. pipiens*, mosquitoes could be forced to become infected by repeated feedings on the same donor.

De Buck (1936) never observed true refractoriness to infection in Dutch anophelines. After one feeding on a suitable carrier he usually obtained 100 per cent. infected. However, he observed an interesting phenomenon, which he termed 'pseudo-refractoriness,' in hibernating *A. maculipennis* var. *messeae*. When induced to feed on a carrier, they showed a much poorer infection than controls (*A. maculipennis* var. *atroparvus*). Normally *messeae* does not feed in winter, and, when forced to take blood, digestion is protracted. De Buck considered that the abnormally slow digestion impeded the migration of the ookinetes, and suggested that variations in the rate of digestion might account for the differences in numbers of oöcysts frequently encountered in the individuals of a batch. Boyd and Russell succeeded in breeding *A. quadrimaculatus* through six generations of brother-sister matings in an attempt to establish a uniformly susceptible strain. They were not successful, and the data which they obtained did not indicate that susceptibility was transmitted as a Mendelian character in this species, as noted by Huff for *C. pipiens*. The fact that the character of the male in each mating is unknown has greatly hampered research of this kind.

In our work, we have observed that a considerable amount of variation in the intensity of infection occurred in all species adequately studied. We have most information about *punctulatus*, and the observations recorded in the following sections were made on this species. We could, however, quote equal variability in *farauti* and *annulipes*, and we know that variation occurred in *amictus*, but the number of this species obtained from any one batch was usually too small for useful comparisons.

We have made observations on (a) variation in degree of infection, and (b) variation in rate of oöcyst development.

#### (a) Variation in Degree of Infection

In a given batch of mosquitoes of the same stock, fed once on a gametocyte-carrier, many specimens were seen with the gut wall packed with oöcysts, whilst in some only a few were present—perhaps only one or two—and in others none at all. This point is illustrated in Table VII, in which the dissections made on two batches of mosquitoes are analysed. Oöcyst counts given in this table are those made when the cysts were well grown, but before the salivary glands were invaded. When many cysts were present, counting them was time-consuming, and in the very heavy infections it was impossible to do so with any pretence at accuracy. Oöcyst counts over 20 are to be regarded as approximations only, and the term 'numerous' refers to counts of the order of 100 or greater.

There is no question, then, that this type of variability is real. Its explanation, however, is another matter, for several factors may operate to produce it, in addition to actual variation in susceptibility of the insect host.

(i) *Amount of Blood Ingested.* There is considerable variation in the amount of blood taken up by fully engorged mosquitoes, large mosquitoes being able to take more than small ones. Mosquitoes may be disturbed before they complete their meal, and this often occurs when the gametocyte-carrier is sensitive to bites and becomes restless. We found, on the average, that fully engorged *punctulatus* took 1.3 c.mm. blood, whereas others with merely a red streak in the abdomen had taken only 0.4 c.mm., i.e., approximately one-third as much. However, our stock of *punctulatus* was very uniform in size, and we endeavoured to pick out only those females which had engorged fully. Occasionally a partly engorged or an unfed mosquito was drawn out in the suction apparatus, but the number accidentally included in this way was very small and certainly insufficient to account for the wide variation constantly encountered.

(ii) *Uneven Distribution of Gametocytes in the Blood.* When the numbers of mature gametocytes in the blood are small, a random distribution might result in detectable variation in the infections produced. When, however, they are numerous, as in many of our observations, significant variation in resulting infections would not be produced unless their distribution in the blood was not random. We know of no evidence to favour an hypothesis of unequal distribution of gametocytes in the peripheral circulation.

TABLE VII  
Variation in gut infection in batches of *A. punctulatus punctulatus* after one blood-meal

Plasmodium	Gameto- cytes per c.mm.	Total dissections				No. of oocysts recorded just prior to sporulation
		Mid-gut		Salivary glands		
		No. dissected	Percentage positive	No. dissected	Percentage positive	
<i>P. vivax</i> ...	1,200	28	96	75	93	3 (recorded twice), 4 (5 times), 5 (twice), 6, 8, 10 (twice), 12, 13 (twice), 15 (3 times), 20 (3 times), numerous (twice)
<i>P. falciparum</i>	850	53	93	114	81	1, 3, 4, 5, 6, 10 (twice), 15, 18, 20 (twice), 25, 30 (3 times), 36, 40, 50, 60, 75, 100, numerous (twice)

(iii) *Hazards Within the Gut.* Mature female gametocytes have to face various hazards between ingestion by the mosquito and encystment in the gut wall. These have been more fully discussed elsewhere (Mackerras and Ercole, in the press), and include: (a) failure to become fertilized—a risk which increases for mechanical reasons with decrease in total gametocyte count; (b) phagocytosis by human leucocytes, which goes on for several hours after the blood is ingested; (c) failure to penetrate the gut wall, the discrepancy between vermicule counts and subsequent oöcyst counts indicating that many zygotes do fail to penetrate successfully.

It is possible that the proportion of vermicules penetrating the gut wall may be related to the rate of digestion, as De Buck suggested for Dutch anophelines. We have no experimental data bearing directly on this point, but we noticed that the rate of digestion did vary in mosquitoes kept under identical conditions. Usually the gut was quite empty

72 hours after feeding, but, in a batch which was being dissected at intervals to study ovarian development, about 25 per cent. of the mosquitoes still had undigested blood present at 72 hours. In our experience, vermicules usually began to penetrate the gut wall at 26 hours, and tiny oöcysts were readily recognizable at 48 hours. However, it is conceivable that, if the amount of blood present at 48 hours is large, many vermicules may be imprisoned in it. As well as differences in rate of digestion, there may be physical or chemical differences, e.g., in viscosity or pH, difficult to demonstrate but important to the welfare of the parasite.

(iv) *Variable Viability of the Parasite.* It has been shown in a previous section that many parasites die, even after they have succeeded in penetrating the gut wall. There is, then, undoubtedly a steady depletion of numbers from the time of ingestion due to causes within the parasite itself.

Each of the factors considered is a variable one, eliminating more or less of the potentially successful gametocytes. When thousands of mature gametocytes are present, the reduction effected is negligible, and the factor limiting the number of oöcysts developing must be the area of gut wall available. However, when only a few mature gametocytes are present, variation in the number perishing between ingestion and encystment may be sufficient to account for some of the observed differences—for example, the difference between a light infection and none at all.

#### (b) *Variation in Rate of Development*

While it was usual to find great variation in the size of the oöcysts in individual mosquitoes after a single infecting feed (for example, in one heavily infected specimen the oöcysts varied in diameter from  $12\mu$  to  $37\mu$ , and in another, from a different batch, from  $17.5\mu$  to  $51\mu$ ), marked differences in the rate of growth of *all* oöcysts were sometimes observed in different mosquitoes in the same batch. For example, the oöcysts in one mosquito measured between 45 and  $53\mu$  in diameter, whilst in another mosquito of the same age, fed on the same donor at the same time and kept under the same physical conditions, none of the oöcysts exceeded  $14\mu$  in diameter.

The former type of variation is probably an expression of the inherent variability of the parasite, but the latter type, where there is a definite retardation of the growth of all cysts, must be an indication of some kind of resistance by the host, although at the moment we are unable to offer any suggestion as to its possible nature. It is noteworthy that a somewhat similar phenomenon has been recorded in helminth infestations.

When all the facts are taken into consideration, there is still an appreciable amount of variability not accounted for by other factors, and variation in individual susceptibility seems to be the only reasonable explanation for it. A mosquito population might, then, be divided into highly susceptible, moderately susceptible, slightly susceptible, and insusceptible individuals. With *punctulatus*, insusceptible individuals must be extremely rare, if they exist at all, for it was common to find all mosquitoes infected when carefully selected gametocyte-carriers (both *P. vivax* and *P. falciparum*) were used (examples 1, 2, 3, 11, 12, 13, 14, in Table VIII).

When numerous mature gametocytes were ingested, the majority of mosquitoes became very heavily infected, and then only one or two classes were discernible—highly and moderately susceptible (examples 1, 2, 11, 12, 13). When fewer mature gametocytes



were taken up, some mosquitoes became heavily infected, some moderately, and some lightly, and some escaped infection altogether (examples 4, 5, 6, 7, 15, 16 and 17). These negative mosquitoes almost certainly belonged to the slightly susceptible group and not to an insusceptible group. Their escape from infection was due purely to chance, and under other circumstances they would have become at least moderately infected. We have repeatedly succeeded at a second attempt in heavily infecting a batch of mosquitoes which had failed to become infected after feeding first on a poor carrier.

It seems reasonable to conclude that in any given population of *punctulatus* all the members are susceptible to infection with *Plasmodium*, but that every grade of susceptibility occurs. If they are fed upon a gametocyte-carrier with numerous infectious

TABLE VIII  
Variation in mid-gut infection in batches of *A. punctulatus punctulatus* after one blood-meal

Example	Plasmodium	Gametocytes per c.mm.	Percentage in each grade of gut infection				
			No cysts	1-5 cysts	6-20 cysts	21-100 cysts	101-300 cysts
1	<i>P. vivax</i>	450	0	0	0	100*	
2	"	960	0	0	4	63	33
3	"	650	0	11	22	56	11
4	"	1,200	4	36	52	8*	
5	"	160	6	26	68	0	0
6	"	260	30	19	32	19*	
7	"	900	31	32	26	11	0
8	"	920	59	33	8	0	0
9	"	400	72	22	6	0	0
10	"	510	96	4	0	0	0
11	<i>P. falciparum</i>	33,500	0	0	0	9	91
12	"	7,400	0	0	0	33	67
13	"	220	0	0	5	78	17
14	"	920	0	17	25	57	1
15	"	850	7	16	28	49*	
16	"	2,100	26	14	23	37	0
17	"	500	28	38	32	2	0
18	"	200	34	45	21	0	0
19	"	830	44	42	14	0	0
20	"	1,960	82	18	0	0	0

\* Several gut dissections were recorded as 'numerous,' and there may have been over 100 oöcysts present.

gametocytes, all will become infected, and it will be difficult to distinguish any gradient at all; with rather fewer infectious gametocytes, the gradient will become apparent; and with a poor carrier, only the highly susceptible individuals will pick up an infection (examples 8, 9, 10, 18, 19 and 20).

#### 4. SUSCEPTIBILITY TO DIFFERENT SPECIES OF *Plasmodium*

We have no evidence to suggest that any species of mosquito may be more or less susceptible to one species of *Plasmodium* than to another. In our experience with *punctulatus* infected with either *P. vivax* or *P. falciparum*, every grade of infection from one cyst to a condition in which the entire mid-gut was densely covered with oöcysts has been observed. In the latter instances the cysts were so tightly packed together that it was

felt that it would have been physically impossible for any more to have developed. We consider that the other species adequately studied, namely, *farauti*, *annulipes* and *amictus*, are also equally susceptible to both parasites. The single batch of *punctulatus* fed on a *P. malariae* carrier was only lightly infected, but this was not unexpected, as the gametocyte count was low.

## 5. TRANSMISSION OF INFECTION

Complete proof of the susceptibility of any species of mosquito to *Plasmodium* requires the transmission of the infection back to its vertebrate host. We have repeatedly transmitted *P. vivax* and *P. falciparum* with *punctulatus* and with mixed cultures of *farauti* and *annulipes*. We have also transmitted *P. vivax* with *annulipes* to five volunteers, and *P. malariae* with *punctulatus* to one volunteer. In some batches of *farauti* and *annulipes*, specimens of *amictus* and *bancrofti* with infected salivary glands were also present, and may have played some part in transmission. However, they were never abundant enough to be used in pure culture.

## V. DISCUSSION

James (1926) was long ago impressed with the epidemiological significance of his studies of laboratory transmission; and our experience with a wider range of species, although undertaken for the purely practical purpose of discovering the most useful one to use in the laboratory, has proved, in fact, to be a study of epidemiology in miniature. I. M. Mackerras (1947) has already discussed this subject in some detail, using both the laboratory and the field data, but it seems useful here to elaborate the findings more fully from the laboratory point of view. We may, however, omit any consideration of the relative infrequency of good gametocyte-carriers and of the conditions which influence their production, for they are discussed elsewhere (Mackerras and Ercole, in the press).

Considering only the mosquitoes, nine species (including subspecies) were listed by I. M. Mackerras (1947) as sufficiently abundant in Australasia to be potential general or local vectors. We have studied seven of them. Five characteristics which influence an anopheline's ability to transmit malaria were also listed, all of which we have examined. It is of interest, therefore, to analyse our findings in relation to each of these characteristics, for they show some interesting analogies and differences between laboratory and field.

*Abundance*, in relation to the laboratory, simply means availability in large numbers. Here *punctulatus* stood out, for it colonized readily when suitable cages were provided (Mackerras and Lemerle, in the press), and so was always available in more than adequate numbers. It was, in short, the most adaptable species to the environment offered. Others might have done as well under different conditions, but we did not discover what those conditions were. Their availability depended on their accessibility in the field, which meant that only *farauti* and *annulipes* could really be regarded as 'abundant' from our point of view, though *amictus* and *hilli* might have qualified had we collected them intensively.

*Susceptibility to infection* was definitely not a major factor in influencing usefulness in the laboratory. All the common species tried were highly susceptible, and our data were not sufficient to discriminate between them. Moreover, in these species we saw no evidence of insusceptible strains, and, although there were individual differences in susceptibility, we were left with the strong impression that they were of less importance

than the other, extrinsic, variable factors influencing intensity of infection. Only in two instances did we obtain evidence of relative insusceptibility, and both were rare species which were tested only for curiosity, namely, *longirostris* and *stigmaticus*.

*Association with man* does not enter as a factor into the laboratory study, for it was promoted by every means in our power, but *avidity for human blood* proved to be most important. Here again *punctulatus* stood out by reason of the readiness with which it would feed, while the other species were at best fickle biters. A few batches of *farauti*, however, fed better than any of the *annulipes* which we used.

Finally, *longevity* also proved to be most important, perhaps more important than willingness to feed, for it was most disheartening laboriously to feed hundreds of mosquitoes and at the end of the developmental period to find only a dozen or so survivors with sporozoites in their glands. It was in this character that *punctulatus* first impressed itself on us, and by reason of which we were ultimately able to maintain the large-infected stocks that were needed. The factors found to favour longevity—namely, a humid atmosphere, temperatures of between 70° and 75° F., and freedom from disturbance—are probably as significant in nature as they were in the laboratory.

To sum up, *punctulatus* proved outstanding as a laboratory insect, because it bred readily in cages, became heavily infected with *Plasmodium*, fed well, and was long-lived. The other species were not as good, except in their susceptibility to infection.

So far, the laboratory findings agree with those in the field, for I. M. Mackerras adduced evidence to indicate that *punctulatus* was the most dangerous vector in the Melanesian region. They agree, too, in the poor vector qualities of *longirostris* and *stigmaticus*, and in allotting considerable value to ability to colonize the local environment freely. They differ, however, in two respects.

In the first place, field evidence would place avidity for human blood as the first attribute of a vector species, whereas to us longevity was even more important. This may well have been partly because we were prepared to spend considerable time in coaxing our anophelines to feed—which would certainly not occur in nature—and partly, too, perhaps, because it has never been possible to study longevity at all adequately in the field.

Secondly, while our assessment of *punctulatus* and of the rare species is in accord with field experience, we saw no laboratory evidence to discriminate between *farauti* and *annulipes*, although the former is an important natural vector while the latter is not. The differences between these two must be of a more subtle kind and involve characteristics which did not come within the scope of our laboratory studies. Certainly our findings support the view that *annulipes* could transit malaria readily enough when presented with favourable conditions for doing so.

## VI. SUMMARY

1. The methods employed in rearing Australasian anophelines for the transmission of Melanesian strains of human malaria are described, and observations are recorded on their behaviour in captivity and susceptibility to infection.

2. *A. punctulatus punctulatus* was more avid for human blood than any other species studied. All would feed on man, but *A. stigmaticus* and *A. longirostris* only with reluctance.

3. *A. punctulatus punctulatus* was also the most long-lived species. Factors favouring longevity were a humid atmosphere, temperatures of about 70–75° F., a constant supply of

carbohydrate food, not less than 6 cubic in. of space per mosquito, and freedom from disturbance.

4. *A. punctulatus punctulatus* from New Guinea was highly susceptible to *P. vivax* and *P. falciparum*. Complete development of *P. malariae* was also observed in it in one experiment.

5. The three mainland species which were most readily available, *A. punctulatus farauti*, *A. annulipes* and *A. amictus amictus*, were highly susceptible to *P. vivax* and *P. falciparum*. Complete development of both these parasites was observed in *A. bancrofti* and in *A. stigmaticus*, although the latter may be rather less susceptible than the other species mentioned above.

6. *P. vivax* in the oöcyst stage was observed in *A. meraukensis*, and *P. falciparum* in the same stage in *A. amictus hilli*. Neither of these mosquitoes was abundant enough for further tests.

7. Complete development of *P. falciparum* was observed in one specimen of *A. longirostris* from New Guinea, but a *P. vivax* infection died out in the oöcyst stage in a small number examined, although development occurred normally in a control batch of *A. punctulatus punctulatus*.

8. Considerable individual variation in susceptibility to infection was found in all species, and was most carefully studied in *A. punctulatus punctulatus*. It is considered that totally insusceptible individuals are extremely rare, if they exist at all, in this race, but that every grade of susceptibility exists. In a single experiment we failed to infect *A. punctulatus farauti*, *A. annulipes* or *A. amictus amictus* with a strain of *P. vivax* from Syria.

9. Our findings agreed fairly closely with field experience, *A. punctulatus punctulatus*—an important vector in the region—proving most useful in transmission-experiments because of its avidity for human blood, long life, and high susceptibility to infection. However, our experiments did not show any marked differences between *A. punctulatus farauti* and *A. annulipes*, whereas in the field the former is an important vector and the latter only transmits malaria sporadically.

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# OBSERVATIONS ON ONCHOCERCIASIS IN THE BAHR-EL-GHAZAL PROVINCE OF THE SUDAN

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The occurrence of *Onchocerca volvulus* in the Sudan was first reported by Bryant in 1932. Studies by Bryant (1933, 1935), Cruickshank (1934) and McKelvie (1935) directed attention to a form of endemic blindness in which singular and apparently specific changes were often seen in the fundus, although externally the eyes appeared normal. The geographical distribution of this condition corresponded with that of *O. volvulus*, and after other possible causes had been excluded it was regarded as a hitherto undescribed manifestation of onchocerciasis. Subsequently, onchocerciasis became the subject of extensive studies in adjacent regions in central and east Africa, and in 1939 the *East African Medical Journal* published a symposium on the subject, which included the records of two cases of filarial blinding in Europeans in Kenya. The present communication records observations in the areas of the Sudan in which Bryant, Cruickshank and McKelvie worked.

## THE VECTOR

Two vectors of onchocerciasis have been incriminated in Africa, *Simulium damnosum* and *S. neavei*. *S. neavei* has not been identified in the Sudan, while *S. damnosum* is widely distributed throughout the areas of that country in which onchocerciasis and endemic blindness occur. For the present, therefore, it may be presumed that *S. damnosum* is the principal vector of onchocerciasis in the Sudan. *S. damnosum* has a well-marked seasonal incidence in the Bahr-el-Ghazal. The Father Superior at Raffile Mission, who has been many years resident there, supplies the following information about *Simulium* in that locality. The flies occur at the river all the year round. Very few are encountered in the dry weather, and these do not fly far from the river's edge. *Simulium* is abundant during the rainy season, June, July and August being the months of greatest prevalence, and at that time the flies are commonly found biting as far as one kilometre from the river. The explanation of this well-marked seasonal prevalence is obscure, but it is probably related to the remarkable rise and fall in the volume of the rivers which occurs during the year. Elderly people in the infested centres maintain that, in addition to seasonal prevalence, the abundance of *Simulium* is subject to cyclical variations extending over a period of years. In other areas of the Sudan Lewis (1948) reports that there appears to have been an abnormal prevalence of *Simulium* in certain years.

It is commonly held that people become infected while fishing in the rivers, but this is doubtful. Fishing is largely a seasonal activity, and is carried out principally by traps and poison when the rivers are low or have ceased to flow. *Simulium* is not prevalent at that time, while during the *Simulium* season, when the rivers are flowing rapidly, there is not much fishing. It has been suggested that the more constant contact with the few remaining *Simulium* at the river counterbalances the comparative scarcity of vectors during the fishing season, but this has not been proved.

### ONCHOCERCA SURVEYS

The technique generally recommended for the demonstration of *O. volvulus* microfilariae is to excise a small piece of skin, to leave it for two hours in a small quantity of saline in a Widal tube, and then to centrifuge and examine the deposit microscopically for living microfilariae. In the present investigation the use of preserved and stained smears was found more convenient. Small skin sections were cut with safety-razor blades and impression-smears were made of the tissue fluid from the cut surfaces. These were dried and later stained by Leishman's method for examination. With parallel tests on the same individuals during the first experimental survey at Basia, it was found that the use of dried and stained films revealed microfilariae as frequently as the technique of centrifuging in saline and examining the deposit for live worms. The stained-film method has the great advantage that microscopic work and the collection of specimens in the field can be carried out independently, enabling the time required in collecting specimens to be reduced to a few minutes per person examined, which is a great benefit in survey work.

#### *Experimental Survey at Basia*

First experiments with the skin-smear technique were made at Basia, near Yambio. This place is in the sleeping sickness area of the Sudan, in which the whole population is subjected to periodic sleeping sickness inspections. Advantage was taken of the fact that the whole population of the area (approximately 800 persons) was assembled at Basia for inspection. Everyone was subjected to a rough clinical examination, and skin smears were taken from all persons showing any abnormalities which might be attributable to onchocerciasis (nodules, keloids, eye changes, skin conditions). Except in the case of those with eye changes, the smears were taken from skin over the site of the abnormality.

Of the total population of 800, 65 persons were found with visible clinical signs which might have been due to onchocerciasis. The incidence of nodules was 2 per cent. Microfilariae of *O. volvulus* were found in the smears from 33 persons, or 50 per cent., of the 65 persons selected. Of these 33 persons, 10 had nodules, 20 had skin abnormalities, and eye conditions were found in seven, of whom five were blind or nearly blind. In seven persons with nodules or tumours resembling onchocercal nodules, the skin was negative for *O. volvulus* microfilariae.

#### *Experimental Survey at the Sueh Rest-House*

The second series of observations was made at the Sueh rest-house, near Tembura. Here again the whole population of the area (approximately 640 persons) was available for examination, and the same procedure was followed as at Basia. The incidence of nodules was 3.4 per cent. Forty persons (6.2 per cent.) were found with visible signs suggestive of onchocercal infection, and skin smears from these persons were positive in 35 cases, or 88 per cent.

At the same time, 44 normal controls were selected from the remaining population, in approximately the same age- and sex-distribution as those showing abnormalities. Examination of skin smears taken from the arm near the insertion of the deltoid of these 44 persons revealed microfilariae in 34 cases, or 77 per cent. This observation suggested that the application of the skin-smear technique to random samples of different populations

might produce interesting results, and in the subsequent surveys this method was adopted. No attempts were made to examine the whole population, but random samples only were obtained, and skin smears were taken as a routine from the arm at the customary site of vaccination.

### *Random Surveys at Mboro and Basilia*

During a visit to the Catholic boys' school at Mboro, skin smears were made by the routine method from the arm of 31 of the pupils, all boys under 16 years of age. On the same day smears were obtained from 40 of the persons found assembled at the inter-tribal court which was sitting at Basilia, close to Mboro. With few exceptions the people from whom smears were made at Mboro and Basilia came from three places, Khor Ganna, Pongo and Peili. The results in persons from these three places are summarized in Table I.

TABLE I  
Surveys at Mboro and Basilia

Place of origin	No. of persons examined	No. positive	Percentage positive
Khor Ganna ...	15, all children under 16	12	80
Peili ... ..	21, all adults	8	33
Pongo ... ..	15, "	12	80

### *Random Survey in the Raffile-Peili Area*

Surveys were next made in the Raffile-Peili area, which has always had a bad reputation for onchocerciasis and blindness. Basing his conclusions on the Christian population, for whom he believes more accurate figures than ordinary government statistics are available, the Father Superior at Raffile Mission estimates the incidence of blindness to be 4-5 per cent. of the population. A similar figure was given many years ago by Cruickshank (1934) for the incidence of blindness in the whole population. Consultation of the hospital records and conversation with local officials suggested that the incidence was heavier in Raffile than in Peili, so it was decided to make two surveys, one at Raffile and one at Peili, each of approximately 50 persons. This was rapidly accomplished in Peili. In Raffile more time was required, during which it was noted that the people living between Raffile Mission and the River Bo showed a much higher incidence of onchocercal nodules than any other population studied, including that on the Peili side of Raffile Mission. The different incidence of nodules in the populations on each side of Raffile Mission was confirmed by clinical examination of larger samples of the populations than are shown in Table II, in which the results of the Raffile-Peili survey are summarized, those on each side of Raffile Mission being shown separately. The results confirm the relatively low incidence of onchocerciasis previously found for Peili people, and give a rough indication of the relative prevalence of this infection in the three localities. The percentage incidence of both nodules and microfilariae diminishes steadily as one moves towards Peili from the rapids where the River Bo joins the River Sueh. Raffile is almost on the edge of the River Sueh, whereas Peili is 13 kilometres from the river.



TABLE II  
Survey in Raffile-Peili area

Locality	No. examined	Positive skin smears, per cent.	Nodules, per cent.	Skin conditions, per cent.
Peili ... ..	32	25	3	6
Between Raffile and Peili ...	20	50	20	20
" " " River Bo ...	38	76	42	10

### Random Survey at Pongo

The next survey was made at Pongo, partly because of the results already obtained at the Mboro boys' school and partly because a survey made in 1946 by Dr. A. R. Hunt indicated the incidence of blindness in this area to be approximately 10 per cent., as contrasted with Father Ruffino's estimate of 4-5 per cent. in the Raffile-Peili area. Dr. Hunt's figure for the incidence of blindness includes Khor Ganna as well as Pongo. The results of the later Pongo survey are summarized in Table III.

TABLE III  
Survey at Pongo

	No. examined	Nodules, per cent.	Positive skin smears, per cent.
Males ...	61	51	90
Females ...	51	40	90

### Survey of Wau Dressers

Finally it was decided to make a survey in Wau, which is in the centre of the endemic area and surrounded by infected foci, but which has not a bad reputation for blindness. Skin smears were taken from 31 dressers in Wau hospital, as they were the only persons in Wau for whom a reliable history of past residence could be obtained. All are recorded as inhabitants of Wau. Microfilariae of *O. volvulus* were found in five cases, or 19 per cent. As any error due to past residence outside Wau would only influence the result by introducing 'false positives,' it is considered that even this small survey shows a lower incidence of onchocerciasis in Wau than in other places from which samples were taken.

## CLINICAL MANIFESTATIONS OF ONCHOCERCIASIS

A few notes are given below on the clinical manifestations of onchocerciasis which were studied in correlation with the *Onchocerca* surveys.

*Onchocercal nodules* may occur on any part of the body, but in the present series over 70 per cent. of the nodules were found at two anatomical sites: (i) the sides of the chest over the ribs, and (ii) the region of the iliac crests and great trochanters. The nodules were rarely as prominent as in the figures shown in the text-books, and never as numerous. Often they were not visible on inspection but were discovered only by

palpation. The youngest person in whom nodules were found was a child aged eight years. Ulceration of the nodules has been observed.

*Skin Manifestations.* The typical skin manifestation of onchocerciasis is xerodermia or pachydermia. The skin is dry, rough and shining, like that of a lizard, and in marked cases appears thickened and elephantoid. In well-marked cases the condition is very noticeable and distinctive, and may be practically universal. In milder degrees the distribution is patchy. During the course of this work many cases were seen with a skin eruption corresponding closely with the clinical descriptions of crawl-crawl, which is now believed—in some instances at least—to be due to onchocerciasis. Many years ago Ensor (1908) recorded seven cases of crawl-crawl in the 12th Sudanese Regiment stationed at Meridi. He noted that scabies was common in the Bahr-el-Ghazal, but states that every care was taken to exclude scabies in these cases, and treatment with sulphur had no effect. The present writer has in the past seen isolated cases of an apparently similar skin condition in the Gedaref and Fung areas, the patients being hunters operating in the almost uninhabited country near the Abyssinian border. Another skin abnormality frequently observed during the *Onchocerca* surveys was a condition of multiple papilloma or verruca. Smears from warts revealed microfilariae in all cases, but so also did smears from apparently normal skin in some of the patients.

*Ocular Conditions—Jur Blindness.* A particularly striking series of 12 cases of blindness was produced by the Father Superior at Raffie Mission on one occasion. All of them had readily detected onchocercal nodules, and 10 of the 12 were elderly persons showing, in addition, extreme degrees of xerodermia. In field conditions complete ophthalmic examination was not carried out. The eyes were merely inspected for gross external and visible lesions, and many persons showed these, conjunctivitis, keratitis, corneal opacity, trichiasis, etc. Even such rough field-observations emphasized, however, that the condition described by Bryant is a very definite clinical entity—a varying period of night-blindness gradually progressing to complete blindness in 6-24 months in a person whose eyes appear normal on casual inspection. Several cases of this type were seen in different stages of the disease, and records in the local hospitals indicate that night-blindness is a usual preliminary symptom. Three cases of established blindness with no external signs were examined ophthalmoscopically in Wau hospital. In two there were changes in the fundus of the type pictured in Bryant's (1935) paper. In the third case the fundi appeared normal; nevertheless the man was blind.

Cases of blindness with no external eye changes were, however, only a small proportion of all the cases of blindness seen. In the remaining cases the eyes showed gross and visible external changes—probably the result of onchocerciasis in most cases. Bryant (1935) has shown, however, that onchocercal keratitis and retino-choroiditis are two distinct conditions, clinically and pathologically. On account of its bilateral nature and the absence of microfilariae in the eye in most cases, it has been suggested that the latter condition is due to a toxin produced by the worms, and that it occurs only in individuals who have become sensitized. This is an important suggestion from the point of view of treatment.

*Other Conditions.* In 10 cases noted during the *Onchocerca* surveys medium-sized lipomata were found. In six instances the site of the tumour was over the iliac crests, and in one it was the side of the chest. Some of the standard text-books refer to the occurrence of lipomata in onchocerciasis, and the present series is of interest in view

of the high proportion of cases in which the tumours were found at the sites of election of onchocercal nodules. Onchocerciasis in the African is said to be associated with keloid formation, and in the first experimental survey at Basia some of the persons selected as having nodules or conditions resembling nodules were really cases of keloid. Probably this partly explains the low percentage of microfilariae found in this group, for subsequent observations gave no reason to believe that keloid formation is a suggestive sign of onchocerciasis.

Workers in the Belgian Congo have shown that hydrocoele and elephantiasis of the genitalia may be caused by *Onchocerca* infection. No data were collected on this subject during the present study, as it was considered undesirable to include examination of the genitalia in routine field-surveys of the population. Hydrocoele and scrotal elephantiasis are, however, extremely common in the areas of onchocerciasis in the Sudan, while *W. bancrofti* infection is extremely rare. Cruickshank (1934) made the interesting observation that hydrocoele and scrotal elephantiasis are much commoner in Peili than in Raffile, whereas blindness is much commoner in Raffile than in Peili. The explanation of this is at present unknown.

TABLE IV  
Summarizing the results obtained in the various surveys

Locality	Positive skin smears, per cent.	Nodules, per cent.	Blindness, per cent.
Sueh rest-house ...	80	3.4	Under 1
Raffile-River Bo ...	76	42	4-5 : this probably includes all three localities
Raffile-Peili ...	50	20	
Peili ...	25	3	
Pongo ...	90	46	10
Wau ...	19	—	Reported low

### COMMENTS AND CONCLUSIONS

A review of the various surveys shows that, with the exception of the survey made at the Sueh rest-house, there is apparently some correlation between the incidence of microfilariae in the skin, onchocercal nodules and blindness, as far as estimates of the last condition are available. A summary of the results is given in Table IV. It is clear, however, that everyone who becomes infected with *Onchocerca* does not become blind. Some other factor is required which is at present undetermined.

The results of the survey at the Sueh rest-house call for some comment. They show that in an area of onchocerciasis the incidence of microfilariae in the skin may be as high in persons having no nodules or other signs of infection as in those having onchocercal nodules, and they suggest that only a proportion of the adult worms in the host's body produce cutaneous nodules, the remainder living in some other situation which has not yet been identified. In this survey also, as compared with the others, the high incidence of microfilariae in the skin is in contrast with the low incidence of nodules and of blindness. It may be noted that the people concerned in this survey are Azande,

whereas those in the other surveys belong to other races, suggesting that racial or dietetic factors may possibly be responsible for the differences revealed by the surveys. Bryant and Fairman (1939) have emphasized that different tribes in the Bahr-el-Ghazal vary greatly in their reaction to different drugs and diseases. According to Pridie (1935), the Azande, a tribe of approximately 200,000 people, contains approximately 75 per cent. of the total number of lepers in the whole Sudan. The possible effect of other factors, such as hyperinfestation, has not been adequately studied, however, and much work is still necessary before definite opinions can be stated. According to Strong (1942), the only effective prophylactic measure is mass removal of nodules, which is said to reduce infection of the *Simulium*, and has been much used in South America. In view of the extensive infection of the skin with microfilariae in persons without nodules noted in this paper, it is difficult to believe that removal of nodules will have much effect on the infection-rate in *Simulium*.

Although *S. damnosum* has a wide range of flight, and there are several records of adults being found many miles from any possible breeding-place, all the information collected during the present study indicates that onchocerciasis and filarial blinding are localized in their distribution, even in an area such as the Bahr-el-Ghazal, throughout which the infection is widely distributed. Measures hitherto adopted for the control of *Simulium* have not been very successful anywhere, but the recent claim of Garnham and McMahon (1947) to have eliminated *S. neavei* from an endemic focus of onchocerciasis in Kenya is of great interest. The application of this method to hyperendemic foci might greatly reduce hyperinfestation, and with it, perhaps, most of the serious clinical manifestations of onchocerciasis.

Many of the signs and symptoms of onchocerciasis cause little inconvenience, but treatment is urgently required in cases with early ocular symptoms which, if not arrested, will ultimately cause blindness. No treatment for onchocerciasis has yet been evolved, but several drugs have shown definite and specific filaricidal properties. The results of chemotherapeutic trials in the Sudan will, it is hoped, be the subject of a future communication.

### SUMMARY

1. Onchocerciasis is prevalent in the Bahr-el-Ghazal province of the Sudan, *Simulium damnosum* being the principal and possibly the only vector.

2. With a simple technique using preserved and stained smears for the demonstration of microfilariae in the skin, *Onchocerca* surveys were made in several localities of the Bahr-el-Ghazal, and the results are described.

3. A high incidence of microfilariae in the skin was found in an area of onchocerciasis in persons having no onchocercal nodules or other signs of infection, and the significance of this is discussed.

4. The clinical signs and symptoms of *Onchocerca* infection seen in the Bahr-el-Ghazal region are described.

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# SEASONAL AND ANNUAL VARIATION IN THE INCIDENCE OF TRYPANOSOMIASIS IN GAME

BY

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## INTRODUCTION

A note has already been published on the incidence of trypanosomiasis in game animals shot in tsetse-fly areas of Shinyanga and Ukerewe, Tanganyika Territory (Vanderplank, 1942), in which it was suggested that trypanosomes are more likely to be found in young animals than in adults, and that there is a pronounced seasonal variation. Certain wild animals, such as kudu and giraffe, showed a higher percentage of infection than others. A great deal of further data is here presented, partly in substantiation and partly in disproof of earlier conclusions.

## OBSERVATIONS

The technique employed has already been described (Vanderplank, 1942). The results were obtained from 378 wild animals shot between June, 1939, and June, 1944, including 153 of which the results are given in the previous paper. Of these 378 animals, some 322 were shot in the Shinyanga fly-bush and the rest at Ukerewe peninsula, fly-bush near Musoma, Lake Province, and Kitilala in the Shinyanga district (but outside the 'Shinyanga fly-bush').

The results are summarized in Table I.

Of species in which more than 10 individuals were examined, giraffe showed the highest infection-rate—37 per cent. Most of these animals were infected with *Trypanosoma congolense* or *T. vivax* and there was one doubtful *T. brucei*. Next come roan, eland, zebra, impala and wart-hog, in that order. The numbers of the others are too small to be significant, beyond establishing that certain infections can occur in certain species. Of particular interest is the finding of one elephant out of six with a *T. congolense* (*T. simiae*?) infection. These results are discussed later.

Table II compares the numbers of young and adult animals showing trypanosomes in their blood. In a previously published table (Vanderplank, 1942, Table II) the young animals showed a higher rate of infection than the adults, but the data were insufficient. Now no significant difference is found between the two groups. It is worth noting that in wart-hogs trypanosomes were found only in the young animals and not in adults.

Table III compares the incidence of infections in males and females. The females showed an insignificantly higher rate of infection. There is, perhaps, no reason to expect any differences between the infection-rate in male and female animals, but it is possible that pregnant females may have their resistance lowered and show relapses, and that in some animals the habits of the two sexes may vary in such a way as to expose one sex to the tsetse more than the other.

\* This paper includes data obtained in the Abercorn area of Northern Rhodesia by Mr. W. E. F. Thomson, Field Officer, Tsetse Research Department.

TABLE I

A summary of trypanosomiasis of wild game

Species	No. examined	No. positive	<i>T. brucei</i> and varieties	<i>T. congolense</i> and varieties	<i>T. vivax</i> and varieties	Mixed infections
Impala ... ..	87	14 (16%)	0	13	1	0
Giraffe ... ..	62	23 (37%)	1 ?	18	10	5
Zebra ... ..	32	4 (12%)	0	2	2	0
Roan ... ..	25	5 (20%)	0	4	1	0
Dik-dik ... ..	12	1 (8%)	0	1	0	0
Eland ... ..	22	4 (18%)	1	3	0	0
Duiker ... ..	9	1 (11%)	0	1	0	0
Steinbuck ... ..	6	1 (17%)	0	1	0	0
Thomson's gazelle ... ..	5	0	0	0	0	0
Grant's gazelle ... ..	4	0	0	0	0	0
Reedbuck ... ..	2	0	0	0	0	0
Bushbuck ... ..	2	2	0	2	0	0
Waterbuck ... ..	1	0	0	0	0	0
Uganda kob ... ..	1	0	0	0	0	0
Kudu ... ..	7	4 (57%)	1 ?	4	1	1
Topi ... ..	8	1 (12%)	0	1	0	0
Wildebeest ... ..	9	0	0	0	0	0
Hartebeest ... ..	2	1	0	0	1	0
Jackson's hartebeest ... ..	1	0	0	0	0	0
Kongoni ... ..	4	0	0	0	0	0
Klipspringer ... ..	2	0	0	0	0	0
Buffalo ... ..	2	0	0	0	0	0
Wart-hog ... ..	37	4 (11%)	1	3	1	1
Bush-pig ... ..	3	0	0	0	0	0
Elephant ... ..	6	1 (17%)	0	1	0	0
Rhinoceros ... ..	1	0	0	0	0	0
Hippopotamus ... ..	3	0	0	0	0	0
Carnivores (leopard 1, lion 2, serval cat 2, genet cat 1) ... ..	6	0	0	0	0	0
Hyena 5, hunting dog 2, and jackal 1 ... ..	8	0	0	0	0	0
Baboon and monkey ... ..	5	0	0	0	0	0
Ostrich ... ..	1	0	0	0	0	0
Porcupines ... ..	3	0	0	0	0	0
Total ... ..	378	66 (17.5%)	2 and 2 ?	54	17	7

TABLE II

Comparison between the numbers of young and adult animals found to be infected

Species	Young		Adults	
	No. examined	No. positive	No. examined	No. positive
Impala ... ..	11	2 (18%)	76	10 (13%)
Giraffe ... ..	3	1 (25%)	59	21 (36%)
Wart-hog ... ..	17	4 (23%)	21	0
Others, including carnivores ... ..	19	2 (10½%)	154	22 (14%)
Total ... ..	50	9 (18%)	310	53 (17%)

Table IV shows the bi-monthly analysis of results (based on animals shot in the Shinyanga fly-bush only), and agrees with those previously published, except for the September-October period. This variation is most probably due to the variation of climate from year to year in this period. These results are discussed later, in reference to the numbers of tsetses found infective and the fly populations.

TABLE III  
Comparison between the numbers of male and female animals found to be infected

Species	Males		Females	
	No. examined	No. positive	No. examined	No. positive
Impala ... ..	54	7 (13%)	34	6 (18%)
Giraffe ... ..	39	15 (38½%)	23	7 (30%)
Eland ... ..	17	3 (18%)	5	1 (20%)
Roan ... ..	11	2 (18%)	14	2 (14%)
Zebra ... ..	19	2 (10%)	13	2 (15%)
Other antelopes ... ..	55	5 (9%)	22	8 (36%)
Wart-hog ... ..	22	2 (9%)	17	3 (18%)
Bush-pig } ... ..	4	0	3	0
Rhinoceros } ... ..				
Hippotamus } ... ..				
Elephant ... ..	4	1	2	0
Others, including carnivores ...	18	0	4	0
Total ... ..	243	37 (15.4%)	137	29 (21.0%)

The difference between the totals is not significant by the  $\chi^2$  test.

TABLE IV  
Showing the distribution of infections according to bi-monthly periods. Denominator shows the number examined, numerator shows the number found positive

Species	Jan.- Feb.	Mar.- Apr.	May- June	July- Aug.	Sept.- Oct.	Nov.- Dec.	Total
Impala ... ..	3/18	2/5	3/7	3/26	0/23	2/6	85
Giraffe ... ..	1/11	6/6	4/7	6/19	1/14	4/5	62
Wart-hog ... ..	0/5	0/1	1/9	0/5	1/9	2/7	36
Others, excluding carnivores	2/11	3/20	3/18	4/27	4/32	4/16	124
Total ... ..	6/45 (13.6%)	11/32 (34.4%)	11/41 (26.8%)	13/77 (16.8%)	6/78 (7.7%)	12/34 (35.3%)	307
Previous results* ... ..	19.0%	58.3%	22.2%	14.9%	27.3%	40.0%	

\* See Vanderplank (1942).

Table V is of particular interest, since it shows for the first time that the trypanosomiasis incidence varies from year to year in the same area. In 1940 it was 43 per cent., in 1941 22 per cent., in 1942 20 per cent., and in 1943 9 per cent. Over the same period a decrease was recorded in the numbers of infective tsetses present, though the tsetse population remained relatively stable.



Table VI and Appendices I-IV\* record the numbers of non-teneral male and female *Glossina swynnertoni* and *G. pallidipes* infective with *T. brucei*, *T. congolense* and *T. vivax* in the Shinyanga area from July, 1941, to December, 1944. Male *G. swynnertoni*, which feed more or less at random on the larger antelopes, giraffe and rhinoceros (only two to three in the area concerned), show a decrease in infection-rates from 1941 to 1944. For the relative numbers of game in the main part of the area, Block 9, see Harrison (1936).

TABLE V  
Showing annual variations in numbers of animals infected

Year	Group I		Group II		Group III		Group IV		Group V		Group VI		Total, excluding Group VI	
	Impala		Giraffe		Roan, eland		Hippo, rhino, elephant, wart-hog, bush-pig		Other antelopes and zebra		Carnivores, primates, rodents and porcupine			
	No. examined	No. positive	No. examined	No. positive	No. examined	No. positive	No. examined	No. positive	No. examined	No. positive	No. examined	No. positive	No. examined	No. positive
1938-39							1	-	3	-	2	-	4	-
1940 ...	13	5 (39%)	4	2 (50%)	4	1 (25%)	6	3 (50%)	10	5 (50%)	1	-	37	16 (43%)
1941 ...	37	4 (11%)	23	11 (48%)	13	2 (15%)	20	-	17	7 (41%)	8	-	110	24 (22%)
1942 ...	31	4 (13%)	18	7 (39%)	15	4 (27%)	11	1 (9%)	27	4 (15%)	2	-	102	20 (20%)
1943 ...	3	-	17	2 (12%)	12	1 (8%)	1	-	13	1 (8%)	5	-	46	4 (9%)
Total and average 1938-43 ...													299	64 (21.4%)

TABLE VI

Numbers of non-teneral males and females infective with *T. brucei*, *T. congolense* and *T. vivax*. Full analysis is given in Appendices I-IV (which may be consulted on application to the author)

Year	<i>G. swynnertoni</i>				<i>G. pallidipes</i>			
	Males		Females		Males		Females	
	No. examined	Percentage infective	No. examined	Percentage infective	No. examined	Percentage infective	No. examined	Percentage infective
1941 ...	775	5.5	130	10.7	—	—	—	—
1942 ...	5,457	5.1	1,214	3.8	2,117	5.8	1,428	5.1
1943 ...	5,592	4.2	1,853	3.6	1,893	3.5	1,309	2.6
1944 ...	7,789	3.3	1,823	3.9	2,328	6.7	1,404	5.8
Total	19,613	4.94	5,020	3.94	6,338	5.46	4,141	4.59

Grand total 35,112.

*T. brucei* infection 32, or approximately 0.1 per cent.

It was not possible during war-time to continue the game observations, but the numbers of animals probably increased considerably under rigid protection. Those shot were animals which came outside the boundaries and raided African cultivation along the borders. *T. brucei* infections were slightly less than 0.1 per cent. over the whole 35,112 flies dissected.

\* These appendices may be obtained from the author by anyone interested.

Some data have also been collected on the course of *T. rhodesiense* (presumed similar to *T. brucei*) and *T. congolense* in captive animals. A male and a female bush-pig, kept at Old Shinyanga and used to feed several hundreds of wild tsetse each week, were examined for trypanosomes once every week from the end of 1940 to the end of 1944. The male showed one *T. congolense* per 200 fields of thick smear during one week in January, 1943, and one *T. congolense* per 20 fields during one week in May, 1943, but did not show trypanosomes at any other time. The female showed one *T. congolense* per 190 and 150 fields during two weeks in January, 1943, and 3/200, 2/70 and 1/200 *T. congolense* during three weeks in March, 1943, but none at any other time.

A young bush-pig, 2-4 months old, captured outside tsetse bush, was bitten by a *G. morsitans* infective with *T. rhodesiense* on August 17th, 1942. Its blood was examined by a thick smear every other day for the next two months; only on one occasion were trypanosomes found (1 per 200 fields). It was bitten again by two known infective flies in October but failed to show any signs of infection. A second bush-pig was also bitten by an infective *morsitans* but failed to show trypanosomes, although some of the rats inoculated with 1 c.cm. of blood became positive for *T. rhodesiense*. A third bush-pig gave a similar history.

A very young wart-hog was purchased in November, 1941, and was bitten by a *rhodesiense*-infected *morsitans* on December 22nd, 1941. Its blood was positive (5 per 200 fields) on December 29th and 31st, 1941, but failed to show any trypanosomes after that date. On January 16th, 1942, one of two rats each inoculated with 1 c.cm. of the animal's blood became positive. Up to March 19th, 1943, 14 rats were inoculated at different times with 1 c.cm. of blood but none showed any subsequent infection. Another wart-hog purchased at the same time was bitten by an infective *morsitans*. This animal never showed trypanosomes in its blood, but one out of 19 rats inoculated with 1 c.cm. of its blood became positive. Similarly, a third wart-hog (treated in the same way) did not show trypanosomes in its blood, but three inoculated rats became positive for *T. rhodesiense*.

A number of observations, summarized below, were carried out on other animals, particularly antelopes. Eland shows *T. rhodesiense* for several days at a time, up to six weeks, after an infective bite. For a further period, rats inoculated with their blood become positive, after which the infection is too scanty to detect. In one instance, however, a goat inoculated with 50 c.cm. of blood from an eland infected two years previously became positive. It has also been found possible to reinfect or superinfect these animals with the same strain of trypanosome.

Impala also appear to throw off this infection after a relatively short period, but are subject to relapses. They can also be superinfected with the same strain.

Reedbuck take rather longer than impala to 'lose' the infection, but otherwise behave in a similar way.

Duiker show the infection periodically, without succumbing, but Thomson's gazelle show trypanosomes in the blood continuously until death, which varies between four months and two years.

Even animals such as the ant-bear, which is very susceptible to trypanosomes, only show *T. rhodesiense* for short periods of a day or so. The two animals used in experiments died from the disease within two months of the infective bite.

I attempted to infect several baboons by infective bites (*T. rhodesiense*) of *G. morsitans*

and by inoculating large numbers of living trypanosomes, but none ever showed an infection.

Infective flies were also fed on four porcupines (a favoured host of *G. pallidipes*); only one of them showed trypanosomes in the blood, and then only on three consecutive days, after which it remained negative.

On the other hand, small carnivores, such as the Fennec fox and jackal, show numerous trypanosomes in their blood and die in a matter of days or weeks.

The populations of *G. swynnertoni* and *G. pallidipes* vary with the season. Apparent densities were measured by means of a standard round and technique, the numbers being estimated in relation to activity. Activity of the flies varies with the climatic conditions, as does the hunger-cycle (Vanderplank, 1947). However, the apparent densities as shown by fly-rounds does indicate the numbers likely to attempt to feed, although the majority of males which appear to the catchers are seeking females and not food.

The numbers appearing to the catchers (apparent density) are highest during the mid-dry season (June-July) and lowest during the early rains (November-December). The actual population is lowest at the end of the dry season (October-November) and highest at the end of the rains (April-May). There is also a yearly variation in fly-density, and the mean numbers of male *G. swynnertoni* per 10,000 yards for the whole of the control area (Block 9, whence the majority of the animals were shot) were, for the respective years 1939-44, as follows: 45, 95, 95, 76, 89, 195.

From November, 1940, to July, 1943, any fully gorged *G. swynnertoni* caught on the fly-rounds in the Shinyanga area were brought into the laboratory, where the blood-meal was extracted and smeared on a glass slide. A sample of 50-100 erythrocytes was measured and the average was taken. It is possible from these averages to classify them into certain groups of animals (Jackson and Vanderplank, 1942). Some 1,600 flies were examined during this period, and the results were analysed month by month. The figures were, however, generally inadequate for a statistically sound monthly analysis, but the total results showed an average of 28 per cent. with erythrocytes measuring between 2.7 and 3.6 $\mu$ , which might come from three species of animals—impala, sheep and goat. Impala were numerous in the area, and sheep and goat frequented the boundaries of the fly-bush, though they very rarely entered them. Seventeen per cent. had erythrocytes measuring between 3.7 and 4.6 $\mu$ . This group includes roan antelope 4.0-4.4 $\mu$ , wildebeest 4.3 $\mu$ , topi 3.8-4.4 $\mu$ , and possibly, along the edges of the bush, Thomson's gazelle 4.2-5.0 $\mu$ ; also dik-dik 4.6 $\mu$  upwards, and giraffe 4.3 $\mu$  upwards. Forty-two per cent. of the tsetse came in group III, having blood-meal erythrocytes measuring between 4.7 and 5.7 $\mu$ . This group includes giraffe 4.3-5.6 $\mu$ , eland 4.6-5.7 $\mu$ , greater kudu 5.0-5.7 $\mu$ , zebra 5.0-5.4 $\mu$ , duiker 4.9-5.4 $\mu$ , steinbuck 5.3-5.4 $\mu$ , klipspringer 5.0 $\mu$ , dik-dik 4.6-5.3 $\mu$ , civet cat 5.5 $\mu$ , leopard 5.4 $\mu$ , and possibly reedbuck, 4.8-5.9 $\mu$ , and cattle, 4.7 $\mu$ , along the edges, but the latter are very rarely attacked by any number of flies. Nine per cent. came in group IV with erythrocytes from 5.8 to 6.7 $\mu$ , which includes bush-pig 6.3 $\mu$ , wart-hog 5.9-6.7 $\mu$ , rhinoceros 6.4-6.7 $\mu$ , wild-cat 6.1-6.3 $\mu$ , and monkey 6.6-7.9 $\mu$ . Only 4 per cent. came from the last group, V, with erythrocytes from 6.8 to 9.0 $\mu$ , which includes man 6.8-7.5 $\mu$ , baboon (a favoured host) 6.9-7.5 $\mu$ , monkey 6.6-7.9 $\mu$ , porcupine 9.0 $\mu$ , and most of the carnivorous animals.

Mr. W. E. F. Thomson, of our Tsetse Research Department, also collected some data on the trypanosomiasis of game in the tsetse areas of Abercorn, Northern Rhodesia.

The tsetse is *G. morsitans*, but *G. palpalis martinii* also occurs along the lakeside (Lake Tanganyika). *G. morsitans* is very sparsely distributed in this area and has been further reduced by control-measures. Table VII summarizes Mr. Thomson's results.

Bruce *et al.* (1913) published the results of examining some 180 wild animals shot in Nyasaland. Their results gave rather higher infection-rates than were found by Thomson and the present author, but this has been shown above to vary with the seasonal changes and from year to year, and most probably also varies with locality and fly-densities. Other workers have from time to time reported upon the examination of a few animals from different localities, and such data have been collected by Wenyon (1926). Data are still very scanty concerning many species of animals, and a great deal of work remains to be carried out on the subject.

TABLE VII  
Record of trypanosomes in wild animals shot and examined by Mr. W. E. F. Thomson in the Abercorn area, Northern Rhodesia, during 1940, 1941 and 1942

Species	No. examined	No. positive
Zebra ... ..	6	0
Roan ... ..	7	2§ (28%)
Eland ... ..	3	0
Duiker (common) ...	9	0
Reedbuck ... ..	5	0
Bushbuck ... ..	6	0
Waterbuck ... ..	1	1†
Klipspringer ... ..	1	0
Buffalo ... ..	6	1†
Hartebeest ... ..	1	0
Wart-hog ... ..	4	0
Bush-pig ... ..	16	3§ (19%)
Leopard 1, lion 1 ..	2	0
Puku* ... ..	1	0
Blue duiker ... ..	1	0
Cane rats ... ..	3	0
Total ... ..	72	7 (10%)

\* Puku was shot on the Lubu River.

† *T. vivax* and *T. brucei*.

‡ ? *T. brucei*.

§ All *T. vivax*

No *T. congolense* found in any of the above

Recently Fairbairn and Culwick (1946) described a method for distinguishing *T. rhodesiense* from *T. brucei*, but, since this method depends on the measurements of a large number of trypanosomes, it would be necessary to inoculate rats with blood from the shot animals and to obtain sufficient trypanosomes for measurements. In such cases in Africa it would be as easy and more certain to test the strains on human volunteers.

## DISCUSSION

Further data have been collected on the trypanosomiasis of wild animals in tsetse-fly areas and on the seasonal and annual variations of blood infections. It is most probable that all the larger antelopes become infected with all species of trypanosomes soon after birth and tolerate these parasites for the rest of their lives. Under adverse conditions

relapses occur, and superinfections, even with the same strain of trypanosome, are possible.

During the heavy rains (March–April) the numbers showing blood parasites rise significantly, although the percentage of infective tsetse does not show a corresponding rise at the time. As pointed out by Vanderplank (1944*a*) and Glasgow (1946), various blood-sucking Diptera are most numerous at this season and may transmit mechanically between animals in a herd.

*G. swynnertoni* favours the larger antelopes and giraffe as food-hosts, a high percentage of which show trypanosomes in their blood. *G. pallidipes*, which feeds generally in the mornings and evenings, as well as at night (Vanderplank, 1941*b*; Chorley and Hopkins, 1942; Williams, 1943), has marked food preferences, pig being a favoured host (Vanderplank, 1944*b*). Although wart-hog and bush-pig do not show trypanosomes in their blood as frequently as the larger antelopes, *G. pallidipes* has a higher transmission-rate than *G. swynnertoni*. These findings are somewhat contrary to expectations, but there are many factors which influence the transmissibility of trypanosomes. Burt (1946) has shown—and I have confirmed his findings with other species of tsetse—that transmissibility is affected by the temperature at which the pupae are kept prior to the emergence of the adults. Temperature also has a marked effect on the transmissibility of *T. rhodesiense* and *T. congolense* in the adult tsetse *G. morsitans*, *G. swynnertoni* and *G. pallidipes* (Vanderplank, unpublished). Transmissibility of *T. rhodesiense* varies according to the species of vertebrate host infected (Vanderplank, 1941*a*, and unpublished observations).

Considering that less than 1 per cent. of animals showed *T. brucei*, and in view of the difficulty of transmitting this species in the laboratory, it is not surprising that less than 0.1 per cent. of the 35,000 flies dissected were found infective with *T. brucei*. Many other authors in different localities have found higher percentages, but unfortunately no adequate surveys of trypanosomiasis in the wild animals of those areas have been carried out at the same time.

*T. congolense* is more transmissible than the *brucei* group, but is affected by the same external factors. Although there is considerable variation, under identical conditions, when *T. rhodesiense* averages about 1 per cent. transmissibility *T. congolense* averages about 30 per cent. These laboratory findings are substantiated by observations from the field. The average number of tsetse infective with *T. congolense* from the 35,000 dissected was 2.2 per cent., or about 22 times as many as with *T. brucei*.

Because of the inability to establish *T. vivax* in laboratory animals, it has not been possible to obtain any experimental data on its transmissibility; 2.1 per cent. of the 35,000 tsetse dissected had *T. vivax* infections, and this result does not vary significantly from that of *T. congolense*.

It would appear from the available data that every tsetse under the natural conditions found in the Shinyanga area is exposed to an infective meal of each trypanosome species, and that the maximum number that would be expected to become infective at the prevailing climatic (or, at least, thermal) conditions do in fact become so.

## SUMMARY

1. Further data have been collected on the occurrence of various species of pathogenic trypanosomes in the blood of wild animals.

2. Thirty-eight species, comprising 378 individuals, were examined by the author,

and some 17 species (72 individuals) from the Abercorn district, Northern Rhodesia, were examined by Mr. Thomson.

3. A high percentage of giraffe, kudu and roan antelope was found to show trypanosomes in their blood.

4. Only a few individuals were found with *Trypanosoma brucei*.

5. *T. congolense* was found in the blood of an elephant.

6. Carnivores, primates and rodents were all negative, although it is known that some species are very susceptible to all species of trypanosomes.

7. There is no difference between the infection-rates (as shown by the presence of trypanosomes in the blood) of young and of adult animals.

8. There is no difference between the infection-rates of male and of female animals.

9. There is a seasonal variation in the numbers of animals showing trypanosomes in their blood; this is highest during the rains, March-April and November-December.

10. There is also an annual variation in the numbers of animals showing trypanosomes; this correlates with the numbers of tsetse flies found to be infective, and probably depends on the climatic conditions.

11. The results of dissecting some 35,000 male and female *G. swynnertoni* and *G. pallidipes* are given on a monthly and annual basis, showing the separate figures for each sex and species, together with type of infecting trypanosome.

12. Some laboratory data are given on the course of infection of *T. rhodesiense* and *T. congolense* in captive wild animals.

13. The blood-meals of gorged wild *G. swynnertoni* were classified by measuring the erythrocytes; this showed giraffe and the larger antelopes to be favoured by this species of fly.

14. The results, together with other known facts, are discussed from an ecological aspect. Although the number of tsetse flies found infective with *T. brucei* was less than 0.1 per cent., with *T. congolense* 2.2 per cent., and with *T. vivax* 2.1 per cent., it would appear that all the flies are exposed to infection. Under the natural climatic conditions, all the flies that would be expected, on the basis of laboratory observations, to become infective, do in fact become so.

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# STUDIES ON SYNTHETIC ANTIMALARIAL DRUGS

## XIX.—THE EFFECT OF THERAPEUTIC COURSES OF PALUDRINE ON THE RELAPSE-RATE OF VIVAX MALARIA

BY

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This paper deals with the relapse-rate of vivax malaria after certain paludrine dosage régimes. It is divided into two sections: in the first a comparison is made between the effects of a standard mepacrine therapeutic course and of two different dosage régimes of paludrine on the relapse-rate of vivax malaria; in the second the effects are described of a single therapeutic dose of paludrine followed by one weekly dose for six months. For the sake of clarity, the cases in the first section are referred to as the first series, and those in the second section as the second series.

### SECTION I

#### COMPARISON OF THE EFFECTS ON THE RELAPSE-RATE OF VIVAX MALARIA OF THERAPEUTIC COURSES OF MEPACRINE AND PALUDRINE

In the first series, each patient, on diagnosis, was placed in one of three therapy groups. There was no selection of cases, the group in which any patient was placed being determined solely by chance.

The dosage régimes used in the three groups were:

- (a) Group I. Mepacrine: 200 mgm. t.d.s. for 2 days, followed by 100 mgm. t.d.s. for 10 days.
- (b) Group II. Paludrine: 500 mgm. b.d. for 14 days.
- (c) Group III. Paludrine: 50 mgm. b.d. for 14 days.

#### *Clinical Material*

All cases within the three groups were suffering clinically from vivax malaria, parasites being present in the peripheral blood. All except 14 cases had returned from the India-



Burma and Malay peninsula theatres of war; the 14 exceptions were almost exclusively from the Mediterranean area, and were evenly distributed amongst the three groups. A small minority only of all cases had not been on previous suppressive mepacrine. Treatment was withheld until it was evident that the attack was not immediately self-limiting. The courses of drug were given in strict rotation.

Daily blood slides were examined during treatment in hospital, and the usual precautions were taken to ensure that the tablets were swallowed. On discharge, each patient was given six addressed cards, with instructions to return one monthly with details of his health.

Out of the 105 patients originally included in this experiment, seven were subsequently excluded for various reasons, and it was not possible to follow up six of the remaining 98.

### *Immediate Effects of Treatment*

The immediate effects of paludrine treatment were satisfactory and similar to those described elsewhere (Adams *et al.*, 1945), except that in the present series the malarial parasites persisted in the blood for a longer period after paludrine (3.2 days) than after mepacrine therapy (2.0 days). The presence or absence of previous relapses did not alter the rapidity of control of the attack.

### *Relapses*

Only the relapses which occurred within six months of treatment are included in the assessment of the effects of the two drugs. Four recorded relapses occurred outside this period, two being in the group given 50 mgm. b.d. and one in each of the other two groups.

The results of the follow-up are given in Table I. All the relapses occurred in patients from the Asiatic theatre of war.

TABLE I  
Relapses of vivax malaria following paludrine and mepacrine therapy

Therapy	No. treated	Proved relapses	Clinical relapses	Average time to relapse, in days
Mepacrine ...	27	3	5	67
Paludrine 500 mgm. b.d.	35	10	3	66
" 50 "	31	5	4	49

The term 'clinical relapse' is used to denote a pyrexia with subjective symptoms similar to previous attacks, showing an immediate response to antimalarials, no information concerning the presence of parasites being available.

### *Conclusions*

The relapse-rate after the two paludrine therapeutic régimes agrees closely with that obtained by Johnstone (1946) using similar dosage régimes. The number of clinical relapses makes assessment of the treatments difficult, especially in view of the 'malaria consciousness' of many sufferers from vivax malaria, but it would appear that there is no significant difference between the relapse-rates in the three groups of patients.

## SECTION II

## THE EFFECTS ON THE RELAPSE-RATE OF VIVAX MALARIA OF 100 MGM. OF PALUDRINE ONCE WEEKLY FOR SIX MONTHS

The patients in the second series were given a single dose of paludrine on diagnosis, and thereafter 100 mgm. weekly for six calendar months. The initial dose varied from 10 mgm. to 300 mgm., but was usually the higher dose.

On discharge, each patient was given 12 cards and four microscope slides, together with instructions on how to make thick blood films, which were to be sent to us if a relapse was suspected. This method proved highly satisfactory, and only two cases of 'clinical' relapse were reported. Of the original 93 patients all except six kept in contact with us whilst taking the drug weekly. After discontinuing the drug a further eight of the remaining 87 patients defaulted. After the 12th card had been received a letter was sent to each patient asking him to submit blood films should any symptoms recur. No case was followed up for less than one year, the average time being  $13\frac{1}{2}$  months.

*Clinical Material*

The cases were unselected and are comparable to the cases in the first series. All were suffering from clinical vivax malaria and showed parasites in the peripheral blood. Almost all came from the India-Burma and Malay peninsula theatres of war, and about a quarter of them had previously been in Africa. Most had received suppressive mepacrine therapy at some period.

*Results*

Eighty-six cases were reported free from symptoms during the weekly administration of paludrine. One patient developed overt malaria whilst on the weekly paludrine, and, as reported elsewhere (Maegraith *et al.*, 1947), in this case parasites were found in the peripheral blood on the third or fourth day after taking 100 mgm. of the compound. The drug-absorption and blood-concentration curves were normal. The patient was subsequently given 100 mgm. twice weekly for six months and has had no further trouble.

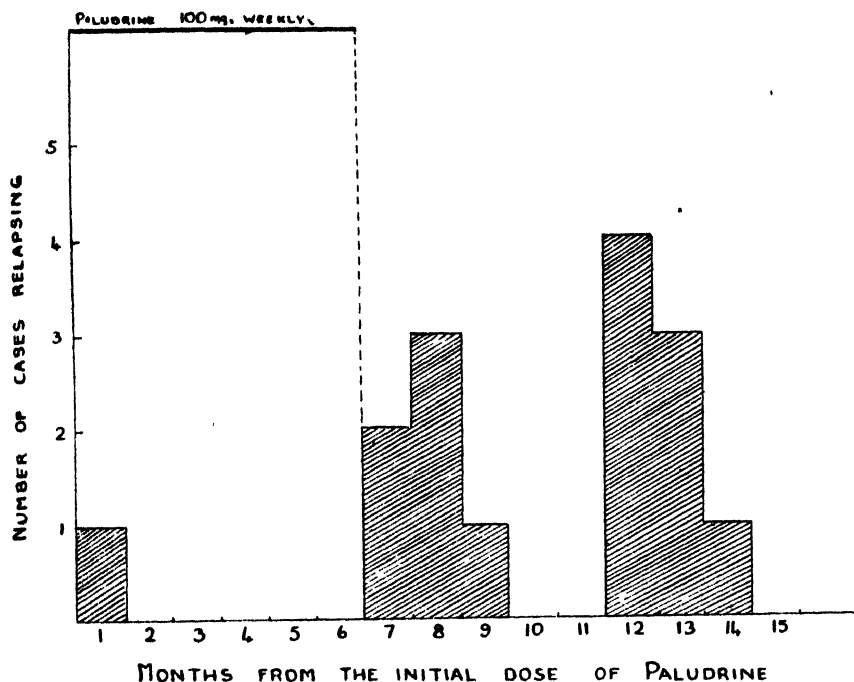
TABLE II

Relapses occurring after taking 100 mgm. paludrine weekly for six months. The two clinical relapses are not included

Total treated	Relapses occurring within 6 months of cessation of therapy	Relapses occurring over 6 months after cessation of therapy	Total relapses
79	11	4	15

In order to compare this series with the foregoing, the relapses occurring over six months from the cessation of therapy are recorded separately. The results are given in Table II. A histogram of the interval between the cessation of therapy and the relapses is given in the accompanying figure. The number of relapses is too few for statistical analysis of the duration of the latent period, but the figure illustrates the long latency

which may occur, and emphasizes the necessity for a long observation-period when assessing the effect of antimalarials on vivax malaria.



Histogram showing the time taken to relapse after a single therapeutic dose of paludrine followed by 100 mgm. once weekly for six months.

Total number treated = 79.

Number of relapses = 15.

### Conclusions

1. Paludrine, in doses of 100 mgm. once weekly, does not eradicate a vivax infection due to the strains from the Far East encountered in this experiment.
2. Although the relapse-rate after a single therapeutic dose of paludrine followed by 100 mgm. weekly is higher than that quoted by Johnstone (1946) following quinine and pamaquin, this form of therapy will be preferred by many patients who are unwilling to enter hospital.

### DISCUSSION

Comparison between the relapse-rate in the first and second series is difficult, for by the time that the weekly paludrine had ceased the patients were a further six months removed from the time of their initial infection.

When the number of the clinical relapses in both series is added to the number of the proven relapses, the observed difference between the relapse-rate of the first series and that of the second is nearly five times the standard error. When only the proved relapses are considered, the observed difference is double the standard error. The diagnosis of the relapses in the second series was better controlled than in the first; the relapse-rate is thus significantly lower in the second series than in the first.

### SUMMARY

1. The effects of two dosage régimes of paludrine were compared with a standard mepacrine therapy in preventing relapses of vivax malaria. No significant difference was observed.

2. The relapse-rate of vivax malaria after a single therapeutic dose of paludrine followed by 100 mgm. taken once weekly was significantly less than after the other therapeutic régimes.

ACKNOWLEDGEMENTS.—Our thanks are due to Dr. J. P. Steel, Medical Superintendent of Smithdown Road Hospital, Liverpool, for his co-operation, to Dr. A. R. D. Adams, many of whose patients are included in this investigation, and to the nursing staff of the Tropical Centre, Smithdown Road Hospital, for their untiring assistance.

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# ON THE CONTROL OF MALARIA IN FREETOWN, SIERRA LEONE

## I.—*PLASMODIUM FALCIPARUM* AND *ANOPHELES GAMBIAE* IN RELATION TO MALARIA OCCURRING IN INFANTS

BY

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(Received for publication September 25th, 1947)

### I. INTRODUCTION

The present investigation, carried out between June, 1944, and June, 1946, was inspired by the desire to obtain an accurate measure of the malaria incidence in Freetown and of the effects of the malaria-control system operating in Freetown at that time.

Malaria control on a large and comprehensive scale was begun in 1943 as an urgent wartime necessity. At that time Freetown was vitally important as the great convoy centre for shipping to and from Africa and the East, and, although the Mediterranean became open to shipping in 1943, traffic to and from the East continued for some time to use the port. Freetown has long been notorious as a highly malarious place, and, although much has been done in the past to reduce the numbers of mosquitoes, sufficient were still present to infect every person three or four times a year, as was clearly demonstrated when large numbers of non-immune Service personnel arrived in 1940 (Turner and Walton, 1946).

Malaria therefore became a serious handicap not only among personnel ashore, but also to some hundreds of the crews and troops on ships at anchor in the harbour, who contracted malaria each month. This constituted a serious threat to the convoy organization.

The problem was investigated between September, 1940, and August, 1941, by Professor D. B. Blacklock (1941), who recommended certain control-measures and the creation of a Mosquito Control Board.

In 1943 the present author arrived in Freetown and initiated intensive civilian control-measures in the city of Freetown and the eastern rural areas. The western rural area was under military control until September, 1945, when it became a civil responsibility. By January, 1944, malaria control was established on a uniformly organized basis, with complete co-operation between the three Services and the civilian departments.

The policy was to rely first and foremost on what are popularly described as 'temporary' antilarval methods, all water likely to provide mosquito breeding-places being treated with larvicide once a week. Any outbreaks of anophelines were located by the extensive use of a network of 'control-houses' or mosquito-catching stations. In this way the hidden breeding-places were traced and eliminated and the localized adult anophelines were killed by pyrethrum-spraying gangs.

To follow the effects of these control-measures the usual measures of sampling human population-groups for malaria infection were undertaken, though it was known that at best they were only roughly qualitative. These observations are being published separately (Walton, 1948).

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Sampling the malaria infection-rate amongst infants can, on the other hand, provide a quantitative measure of the rate of human malaria infection, provided that the extent of congenitally acquired infections is known and that examinations are made at frequent intervals to discover when infection occurs (see Davey and Gordon, 1933 ; Hackett, 1937 ; Turner and Walton, 1946).

As will be shown, Freetown provided unusual opportunities, for the study of malaria transmission was greatly simplified by there being but one species of *Anopheles* and one species of *Plasmodium* among a predominantly African population. This fact should be stressed when considering the present investigation.

Hitherto it has not been possible to correlate the malaria infestation in the mosquito with that in man as a mathematical relationship. In this paper an attempt has been made to do so. Theoretical values of human malaria infestation have been estimated from measures of infective anophelines and have been confirmed by direct observation. Arbitrary values and certain assumptions are used in arriving at this relationship. Nevertheless, it is hoped that this investigation will help to bring nearer a more exact understanding of the relationship between malaria in man and in the mosquito. The numbers of anophelines have been measured at levels believed to be well over 50 times lower than hitherto, and infants have been examined whenever possible at monthly intervals.

## II. FREETOWN AS AN ENVIRONMENT

### 1. *Topography*

Freetown is a city of 8,000 houses which, together with the business- and shopping-centre, lie along a coastal plain up to one mile wide. This plain shelves slightly to the Sierra Leone River. The city is abruptly backed by steep heavily wooded mountains.

Freetown is well isolated from neighbouring highly malarious places, except for the narrow coastal plain on either side. Mosquitoes from outside can reach it along the coast, or be blown by heavy winds from places over five miles away, or be brought in by the numerous forms of transport which radiate outwards in nearly all directions. The city is well supplied by railway facilities, by omnibus and motor-launch services, and by a regular heavy traffic of lorries to and from the Protectorate ; it is also visited by many native sailing-boats.

The coastal plain is largely composed of acid laterite rock, clay, and laterite soil. There is very little humus, as this has been removed by a combination of heavy rainfall, dry-season desiccation and human activity. The growing of sweet potatoes in mounds of soil, and the persistent practice of the sanitary department of removing grass by the roots, are largely responsible for this lack of humus. The lateritic rock absorbs much water, and, owing to its rough and deeply pitted surface, provides multitudes of small pools suitable as breeding-places for *A. gambiae*.

While parts of the city are well planned, there are still many areas where the streets are bare rock and soil, without drains, where the houses are mere shacks, and where the compounds or gardens contain many pools and puddles in the rainy season.

Much is now being done which will alter this state of affairs. Legislation makes it an offence not only to allow mosquitoes to breed, but also to allow conditions to exist which might lead to mosquito-breeding. Compounds are steadily being filled and levelled, roads surfaced and drains provided. Only when this has been completed will

it be possible to eliminate anophelines by the usual methods. Meanwhile, a large and specialized mosquito-control organization is a necessity.

## 2. *The Houses*

The majority of the houses are built of wood or of wood and corrugated iron, some are built of concrete, and a few of mud. Most houses are single storeyed, but some are quite large and have two or three storeys. Roofs are mostly of corrugated iron, but tiles made from folded palm-fronds are also extensively used on the smaller houses. All the houses are subdivided to a greater or lesser extent into small rooms, generally of not more than 1,000 cubic ft. capacity. Owing to the prevalent native habit of renting and sub-renting, locking of doors is extensively resorted to, so that often not more than half the rooms can be entered. The average number of rooms per house may be reckoned as six, and normally two persons sleep in each room.

## 3. *The Human Population*

The human population is stated to be 80,000 (Edge, 1946), of which the descendants of the emancipated slaves, called Creoles, constitute the wealthier and more educated classes. A large population of Temne people, who originally inhabited the area before the settlement in the year 1792, and a slightly smaller population of people of the Mendi tribe from the interior, together with the Creoles, form the bulk of the population. In addition, there are smaller groups of different tribes—Kroo, Sherbro, Locco, Mandingo, Susu, Fulani—as well as Asiatics and a few Europeans.

## 4. *The Climate*

The temperature is constant, varying between 70° and 90° F. in the shade. The rainfall is confined to six months in the year, and is at times very heavy. Humidity is largely influenced by rainfall. The accompanying histograms (figs. 1-3) summarize some available climatic data. Conditions are suitable for the breeding of mosquitoes in every month except possibly April or March, when the humidity is lowest. The long dry season, however, reduces the number of available breeding-places almost to nil, for the rivers become mere trickles, and only in them and in a few wells do the mosquitoes manage to maintain themselves. The production of *A. gambiae* reaches a peak in June and falls during the remaining wet months.

For comparison between past and present conditions in Freetown, and for information on the bionomics of *A. gambiae*, extensive use has been made of papers by many writers, especially by the following: Barber and Olinger (1931), Causey, Deane and Deane (1943), Gibbins (1932), Haddow (1942), Kauntze and Symes (1933), Lamborn (1925), Shannon (1932), Soper and Wilson (1943), Symes (1932), Taylor (1930), Muirhead Thomson (1945, 1947b, 1947c), Tredre (1946), Turner and Walton (1946), and Wilson (1936).

# III. AFRICAN INFANTS IN FREETOWN

## 1. *Factors Arising in the Use of Infants in Malaria Study*

To obtain a reliable measure of the malaria incidence among infants, the group from which blood samples are taken must be unselected. The inclusion of infants attending hospitals or clinics should be avoided, since they may be brought for treatment either

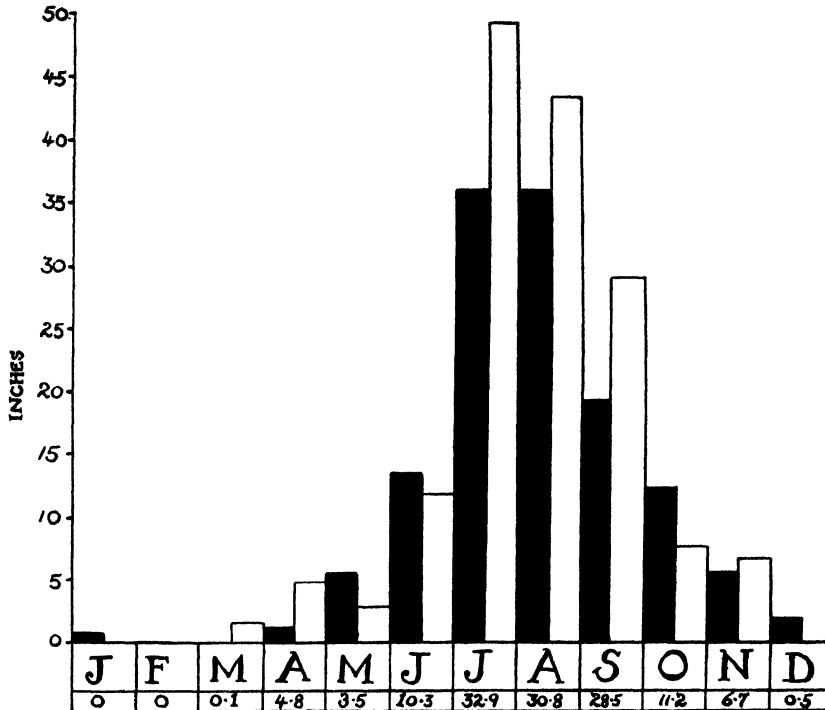


FIG. 1. Rainfall. The black columns show the mean monthly rainfall from 1936 to 1943; the white columns show the same data for 1945, a year of exceptional rainfall. The figures in the bottom row show the rainfall in inches for 1944.

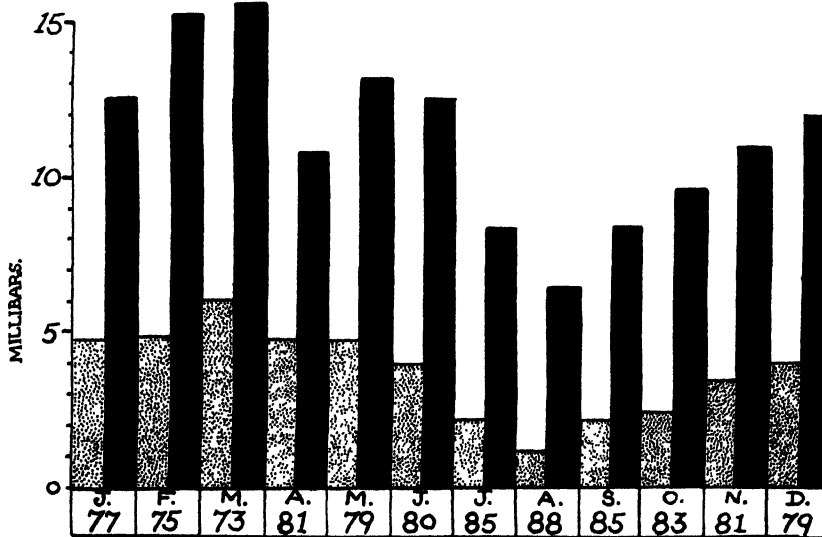


FIG. 2. Humidity: mean monthly saturation deficit for 1944. The stippled columns show values from readings taken at 7 a.m., the black columns from readings taken at 2 p.m. The figures in the bottom row show the mean monthly relative humidities in millibars.



because they have malaria or because they have some other form of sickness which is associated with an abnormal malaria incidence. Similarly, infants of wealthy parents should be omitted, since they may have been provided with mosquito-nets and medical treatment, quinine or mepacrine.

In Freetown, and probably in other large African towns, the human population is abnormally constituted. There is an excess of young and middle-aged males seeking employment and of children who have come for education. It is doubtful if babies under a year old exceed 2 per cent. of the entire population, and, in order to observe 200 infants, as we did, it is necessary to have at hand a total human population of over 10,000.

Since few infants became infected, and since many were lost through death and migration, a large number had to be observed in order to obtain significant numbers of positive observations. As the chance of superimposed infections was small, it was decided

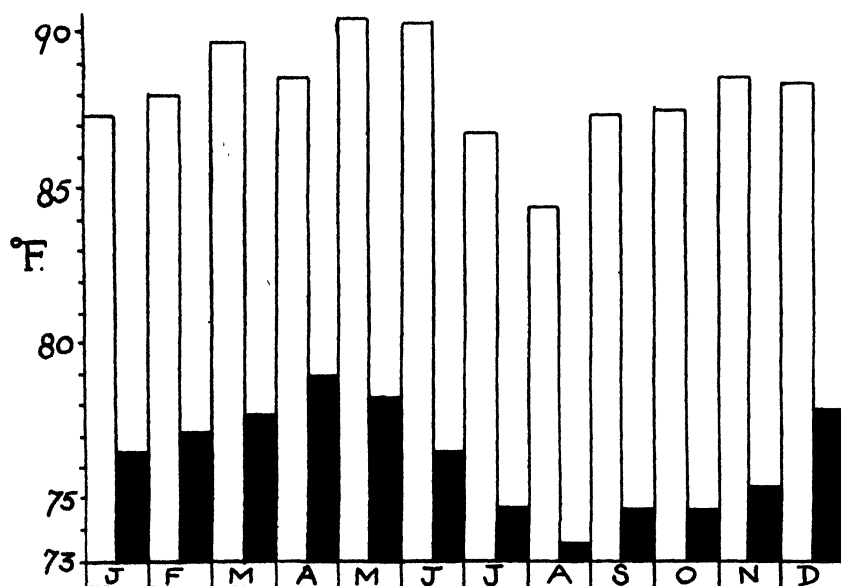


FIG. 3. Temperature: mean monthly maximum and minimum shade temperature in degrees Fahrenheit for 1944. The white columns show maximum values and the black columns minimum values.

to attempt to find the duration of single infections by following up the infants after they became infected. For most practical purposes the rate at which new infections are being acquired by infants is all the information required, but the duration of single infections can only be found when they are acquired quite infrequently; otherwise superimposed infections in the same infant cannot be distinguished from exacerbations or relapses.

## 2. Technique of Obtaining and Examining Blood Samples

An African nurse made both thick and thin films of blood taken from the great toe of the babies. She was taught to make the former of such a thickness that the average field of a 1/12 in. oil-immersion objective contained about 10 leucocytes. Thin films were only used to identify the species of parasite where necessary. All slides were marked with a serial number in blue grease-pencil, to distinguish them from other blood slides

marked with red. The thick blood films were stained with Giemsa and examined by an African medical entomological assistant.

Every 5th to 10th slide was selected at random and checked. As a routine, 150 fields of a 1/12 in. objective were examined on each thick blood film. A single gametocyte (crescent) seen during such an examination was accepted as positive, but less than four trophozoites (rings) were not accepted, though they were recorded. Less than four rings in 150 fields were noted on 12 occasions unaccompanied by crescents, and their significance will be considered in the discussion. All such slides were checked either by the author or by Dr. J. G. S. Turner.

### 3. *Compilation of Data*

For each infant the date of birth, address and name were entered in a register, and a serial number was given. The date of each subsequent visit, the result of the blood examination, and the rectal temperature were recorded. The data were tabulated after the investigation had continued for some time. New infants replacing those lost through various causes were, of course, younger than those already examined. They were grouped in columns according to the date of birth and age. The examination sequences of 37 infants were discarded from the final analysis as being unsatisfactory.

### 4. *The Shifting Infant Population*

Not only are the infants frequently moved about within the city—thus making it difficult to trace them—but they leave the city and visit the country much more often than was at first realized. At one time or another 265 infants were under observation. Of these, 40 are known to have left Freetown during the 18 months of study; three emigrated to Monrovia in Liberia, two went to Bathurst in the Gambia, and eight made journeys of well over 50 miles; 17 died; and nothing is known of the ultimate fate of a remaining 104, who became untraceable.

Of those who left Freetown, six returned after an average absence of two months. Three had crossed the estuary to the Bullom shore. Two had been to Waterloo, 20 miles from Freetown, and one had visited Hastings, 10 miles away. Five of them were infected with malaria on their first examination on return to the city.

During the rainy season fewer infants—generally less than 10 per month—either left Freetown or were untraceable. In the dry season the corresponding number was 20–35 per month, and all the six infants mentioned above as having returned to Freetown were absent between December and April. Of 37 infants who contracted malaria, infection was first noted in 24 during the dry-season months. It appears that infections were rare in Freetown in any month, but that they were often acquired in the country during the dry weather, when the African people travel most.

It is interesting to examine the data on anopheline abundance at this time, to see how much malaria would be expected among these infants had they been confined to Freetown.

## IV. *ANOPHELES GAMBIAE* IN FREETOWN

### 1. *Methods of Collecting A. gambiae*

*A. gambiae* formed at least 99 per cent. of the anopheline mosquitoes captured in houses in Freetown between June, 1944, and June, 1946. *A. funestus* was finally virtually exterminated from the whole area by late 1943. In Freetown *A. gambiae* would be classed

as a domestic mosquito, breeding for the most part in the innumerable small puddles and other small collections of water which occur throughout the city during the rainy season. It probably spends as much of its time in human habitations as any other anopheline; nevertheless Freetown offers a great variety of out-door resting-places, and we do not know what proportion uses them. The civilian malaria-control unit of Freetown was reorganized from 1943 essentially for purposes of control—not for research; and, in order to follow the effects of control and as a guide to antilarval work, a network of 81 catching-stations was established. Every hundredth house in Freetown was a catching-station. They were so spaced as to be about a quarter of a mile from one another, and were selected to produce the maximum number of *A. gambiae*, since most of them were known to be favourite day-time resting-places.

Mosquito collecting was carried out in the same two rooms in each house once every week. The same small gang worked throughout the investigation. White sheets were quietly spread over the floor and furniture, and, with the door and window closed, the room was sprayed thoroughly with a 1 in 64 kerosene dilution of Stafford Allen's 'Pyefly' pyrethrum extract giving 0.1 per cent. pyrethrins. All the mosquitoes which had fallen on to the sheets after 10 minutes were placed in specimen-tubes. These tubes were kept moist by placing small pieces of damp blotting-paper on the bottom and covering these with a cotton-wool plug. No repellent effect of the pyrethrum-spraying was noted. One house—40, Syke Street—has been used as a catching-station in this manner for a number of years. The same two rooms sprayed on 232 weeks still contain anophelines if breeding occurs in the vicinity.

## 2. *The Number of A. gambiae in Freetown*

Table II gives the number of female *A. gambiae* per thousand rooms per month between June, 1944, and May, 1946, and is based on spray-sheet collecting in 162 rooms each week.

These data can be conveniently condensed by stating them as the number of female *A. gambiae* per average room in six-month periods (Table III), calculated by multiplying the mean six-monthly room-density by 182 days.

## 3. *The Anopheline Infection-Rate*

The *A. gambiae* were, almost without exception, captured in the morning and dissected in the afternoon of the same day, and were still alive when dissected. Salivary glands were always examined fresh by lightly crushing them in saline under a very small cover-slip. The information required was the sporozoite-rate of the *A. gambiae* in the houses at the time of capture.

If hand-catching methods had been used, insufficient numbers would have been obtained for dissection to produce significant sporozoite-rates. By using the spray-sheet method in some 30,000 rooms, 2,250 *A. gambiae* females were obtained and dissected, although the mean room-density was very low. For instance, the mean number of female *A. gambiae* in Freetown during the three years 1944–46 was only one in every 10th room per day—that is, roughly one in every second house. The highest room-density observed in any single month was only 0.447. Such a figure was often due to the temporary occurrence of some 10–20 anophelines in only one or two houses among the 80 investigated. Particularly during the 18 months when the infants were being investigated was the mean

room-density very low—only one female *A. gambiae* per 25th room per day; that is, one in every 5th house. Tests were made in which rooms containing many anophelines were each hand-searched by three expert collectors in succession and then immediately sprayed over white sheets by African labourers. On the average the labourers obtained a number equal to those previously caught by the hand-catching experts. Ribbands (1946), working at Aberdeen, near Freetown, found similar results. It is assumed that spray-sheet methods are twice as efficient as hand-catching where anophelines are fairly abundant, and that where they are few the difference in the effectiveness of the two methods must become increasingly great.

Despite the use of this method, the numbers of anophelines in Freetown fell so low during the dry seasons as to preclude the measurement of the sporozoite-rate. In March, 1944, 788 rooms in houses along a stream were tested by the spray-sheet method. Only one female *A. gambiae* was captured.

During the investigation of the infants the proportion of *A. gambiae* infected with sporozoites was falling rapidly. In 1944 the proportion was 5.1 per cent. among 1,296 dissected. In March, 1944, a small collection of 35 from houses along the Alligator River gave a rate of 28.5 per cent. In that month the rate for the whole of Freetown was 15.3 per cent. Similar high rates in *A. gambiae* are reported by Muirhead Thomson (1947b), Barber and Olinger (1931), Soper and Wilson (1943), and Gordon *et al.* (1932).

But in 1945 only 0.4 per cent. of 706 *A. gambiae* dissected had salivary-gland infections, and between July, 1945, and September, 1946, only one was infected among 610.

Since the sporozoite-rate was changing during the period of observation, and since we wish to compare the sporozoite-rate with infections in infants, the proportion of infected *A. gambiae* caught from October, 1944, to March, 1946, is used. During that time the sporozoite-rate of 861 *A. gambiae* dissected was 0.34 per cent. If those dissected in September, 1944, are included, the rate becomes 1 per cent. (10 positives in 922), but this is too heavily weighted.

#### 4. Estimation of Risk of Human Infection

The anopheline infective density (Davey and Gordon, 1933) is a means of expressing the malaria-transmitting ability of an anopheline population. The number of *A. gambiae* female-room-days per six-month season in Freetown is given in Table III. The total number of mosquito-room-days from October, 1944, to March, 1946, is obtained by adding together the number of mosquito-room-days in three seasons. The number of female *A. gambiae* present in each average Freetown room for one day during that time was therefore 21. Of these, 0.34 per cent. were found to have sporozoites in the salivary glands. Therefore the number of infected *A. gambiae* in each average Freetown room from October 1944, to March, 1946, would be  $\frac{21 \times 0.34}{100} = 0.07$ . That is to say, there was

on the average only one infected *A. gambiae* in every 14th room per 18 months. Ignoring the possibility of infective bites being received outside, we assume that these anophelines fed in the room in which they were caught. We also assume that one out of every two of them fed on one person in the room, since it is also assumed that they feed every other night (Barber, 1936; Thomson, 1947b). The average number of persons per room in Freetown was two, and there was one infected *A. gambiae* in every 14th room which fed on one person every 28th night. One in 56 persons would therefore receive an infected

mosquito-bite. But this is the maximum estimated number that could possibly have been infected. Other factors, which would greatly increase this figure, must be taken into account; they are considered in section VIII below. What interests us at this stage is the maximum number of persons whom one would expect to be infected in the broadest sense—this is, less than 2 per cent. of the population in the 18 months from October, 1944, to March, 1946. To see what actually happened we will now turn to the infants once more, and will learn that our expectations fell very far short of fact.

## V. MALARIA IN AFRICAN INFANTS IN FREETOWN

### 1. *Parasite Infestation of Infants*

The tabulated data accumulated between June, 1944, and June, 1946, from 2,191 blood examinations are first treated as a series of random samples of the parasite infestation of infants at different ages. The data are depicted graphically in fig. 4.\* The same data, placed in age-groups of three months and plotted semi-logarithmically, are shown in fig. 7.

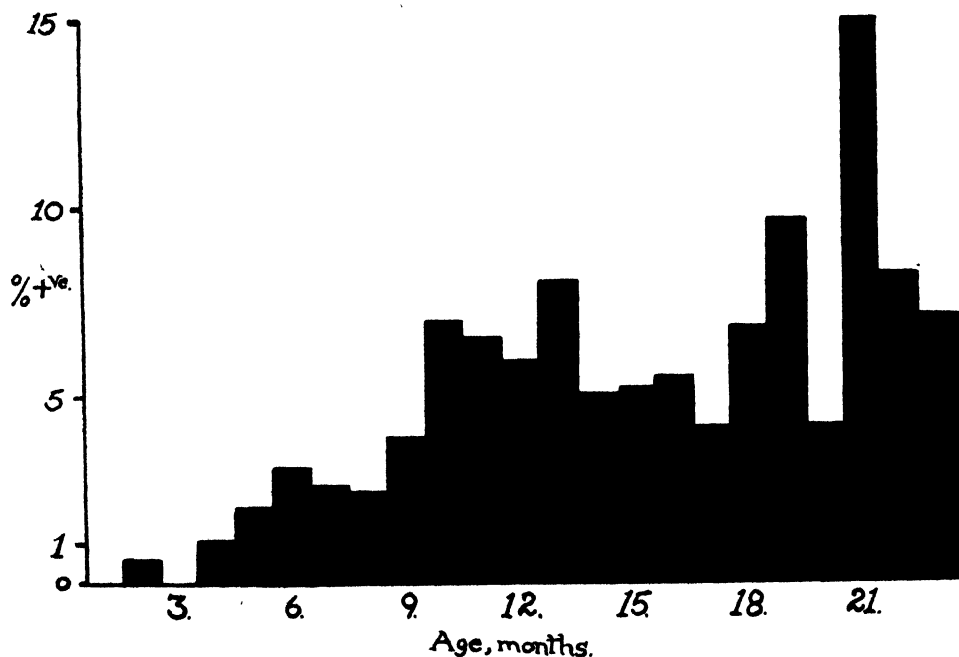


FIG. 4. Percentage of Freetown infants found infected in each month of age.

The curve, which takes a simple form  $\log y = \frac{a}{x} + b$ , where  $y$  = percentage positive and  $x$  = the age in months, does not represent the true form of increase in infection with age. That could only be obtained by studying static native populations.

\* A table showing the original data is deposited in the London School of Hygiene and Tropical Medicine and at the Royal Society of Tropical Medicine and Hygiene.

The data suggest that, under the conditions imposed by mosquito control during 1944, 1945 and 1946, a state of stability is reached when the children are two years old, after which about 6 per cent. would remain infected with malaria. We cannot yet apply this knowledge to adult Africans, since we do not know whether anophelines feed equally readily on infants and on adults.

It is improbable that mosquito control could produce a further reduction of malaria, owing to infections acquired during occasional visits to the surrounding countryside and to immigration.

## 2. *The Effects of Mosquito Control*

Fortunately, from the work of Davey and Gordon (1933), we have some reliable data of the rate at which infants became infected in Freetown at the end of the rainy season of 1931. Davey and Gordon examined 72 infants in Freetown and 82 from the neighbouring village of Kissy. The infants were examined at their homes, and thick blood films were used, so that the information is comparable with the results obtained in the present investigation. From the room-density data, which were obtained by hand-catching methods, it appears that the number of female anophelines present per average room per year in Kissy during 1931 was 2,400, compared with a figure of 14 per year for the 18 months from October, 1944, to March, 1946, in Freetown. Taking into account the different sporozoite-rates found, it is probable that about 5,000 times as many infected anophelines would have been found in Kissy in 1931 as in Freetown in the 1945 period had spray-sheet methods of collecting been used. It is not clear what the density of anophelines in Freetown was in 1931. Davey and Gordon give a graph showing only one anopheline per room in June. Later they compare the numbers found in well-illuminated and in badly illuminated houses and show that in the badly illuminated houses—the only type used in the present investigation—there were 3.5 anophelines per room. It can only be assumed that the spray-sheet wet-season room-density of Freetown in 1931 would have been about 7. The comparable number of infected bites per room per year in Kissy in 1931 and in Freetown in 1931 and 1945 would be roughly 250, 30 and 0.07.

Table IV compares the parasite infestation in the same age-groups of infants from Kissy and Freetown in 1931 with those from Freetown in the 1944–46 period. The same data are shown graphically in fig. 5.

That this marked reduction occurred only in Freetown, and not also in the surrounding country, is shown by blood examinations made in the wet season of 1946 by Davidson (1947). Children and infants were examined in two areas: first in the coastal villages in the Colony south-west of Freetown, Sussex, Lakka, No. 2 River, Hamilton, Ogoo Farm and Baw-Baw; secondly in Magbenkiti, Mafawki, Rochendata, Marampa mines and Lunsar, all about 80 miles north-east of Freetown. Eighty-two per cent. of 368 children aged 2–10 years were infected in the coastal villages, and 77 per cent. of 442 from the villages in the interior. Infants examined were all under six months old. In the coastal villages three were positive among seven examined, and in the Marampa area 17 were positive among 28. The mean age is not known, but the combined rate of infection in 35 infants living outside Freetown was 57 per cent.

• It is interesting to note that, while 6 per cent. of infants were infected in 1945, the corresponding rate of infection in Royal Air Force and European military personnel during the rainy season of 1945 was only 2.5 per cent. (Army in April, 1941, to May, 1942, 104

per cent.) (Turner and Walton, 1946). At the same time malaria ceased to be of any importance among the crews of ships using Freetown harbour.

It will be seen from Table I that in the past the African infant mortality-rate had risen each rainy season, and that, once mosquito control was effectively established, the rate fell to a constant level throughout the year. The reduction seen in the wet season was 27 per cent.

Comparison of the parasite infestation in similar age-groups of infants shows that, with the exception of the age-group 11-22 months, there was a reduction, whether passing from dry season to dry season, from wet to wet, or from dry to wet (Table V).

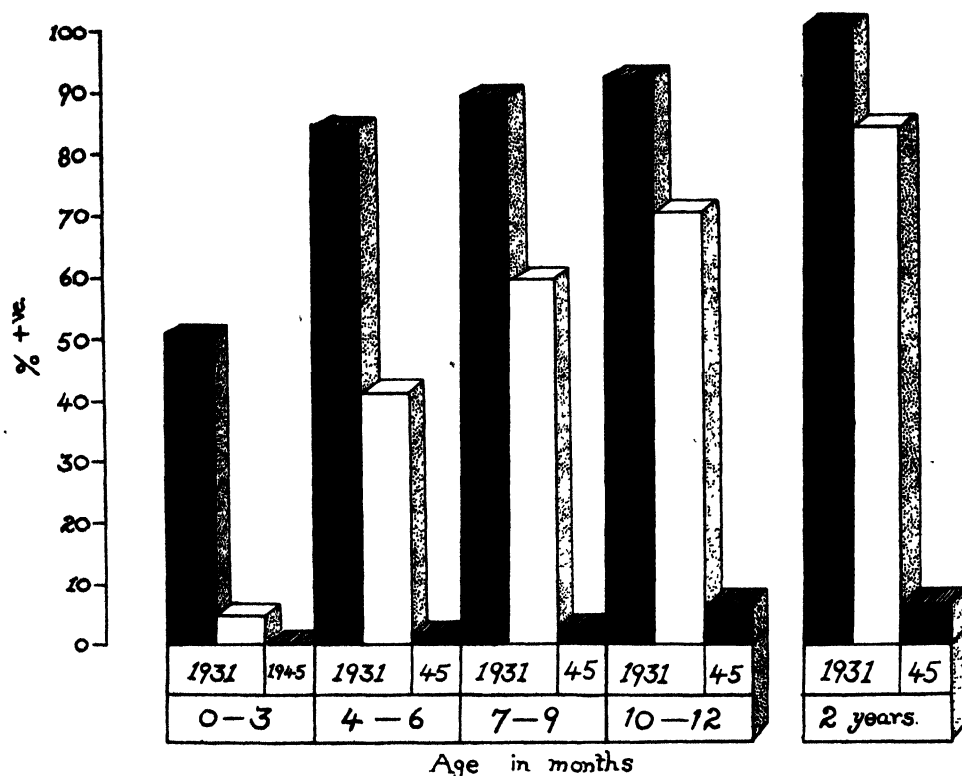


FIG. 5. The effect of mosquito control on the parasite-index of infants at different ages. The tall black columns show the parasite-indices of infants living in the rural village of Kissy, close to Freetown, in 1931; the white columns show the parasite-index of Freetown infants in 1931, and the short black columns show the same for the period 1944-46.

### 3. Rate of Infection of Infants

The data so far discussed have been qualitative rather than quantitative. It is possible, however, to obtain some factual information from the observations, despite the fact that the infants were being missed one month out of four (in almost all cases there were some months in which, for some reason, the infant could not be traced). A few infants were observed for periods up to 23 months, and 50 were followed for 18 months or more. By

grouping these latter together we are able to tell with some accuracy how many infections were acquired during that time.\*

The actual number of blood examinations made was 737. Among these there were 163 omissions. Isolated positive examinations of one month's duration, with previous and subsequent monthly examinations either negative or omitted, occur only six times in 737. Consequently, one would not expect to have missed many such positives among the 163 omissions, especially as all, except one, are clearly part of an infection lasting many months. To make allowance for three such positive findings as having been missed among the omissions would be liberal.

One of the infants—the second in Table XI—appears to have received two infections with different *P. falciparum* strains. It is unlikely that a recurrence or recrudescence could occur with such severity.

Twelve to 15 infections were received by the 50 infants in 18 months—that is, one in four. The infection-index of these infants was 5 per cent. This might give an impression of very little malaria, but that that would be a false impression is clearly demonstrated.

The apparent discrepancy between the low infestation of 5 per cent. and the infection of one in every four infants in 18 months which produced that rate, lies, of course, in the short mean duration of infection and the occurrence during the infection of periods when the parasites are present but are in insufficient numbers to allow of their detection.

#### 4. *Relation between the Anopheline Infection-Rate and the Malaria Parasite-Rate in Infants*

There must be some close relation between the density of infective anophelines and the human parasite infestation. Turner and Walton (1946), assuming that the bites of mosquitoes are made among the human population by a random distribution, and assuming an arbitrary mean duration of human infection, calculated the average parasite-indices† of *P. falciparum* produced in young children by various numbers of infective bites received in a year.

With the statistical help of Major J. L. Harrison these values have been recalculated, using the mean duration of infection derived from the present series of infant examinations. The methods used in estimating the number of infective mosquito-bites and the parasite-indices produced are given in section VIII. The reader is referred to Table VI, which gives the calculated parasite-indices corresponding to a wide range of infective bites.

In section V, 1, it was shown that the maximum parasite infestation of Freetown infants was 6 per cent. From Table VI this would correspond to 0.25 infective bites per person per year. The calculated number of infective bites would be  $0.0047$ , i.e.,  $\frac{0.7 \times 0.04 \times 0.34}{2}$ , which suggests that only one in 200 persons would be infected in Freetown.

We have, in section V, 3, considered 50 infants of whom 15 were infected in 18 months—that is, 0.2 infective bites per child per year. The corresponding parasite-index in the 50 infants can be obtained by expressing the positive observations as a percentage

\* A summary of the examination sequences of these 50 infants is deposited in the London School of Hygiene and Tropical Medicine and at the Royal Society of Tropical Medicine and Hygiene.

† The parasite-index is the number of persons in a group in whom malaria parasites can be found when the blood is examined on one occasion only, expressed as a percentage of the total number examined. It is an approximation to the true parasite infestation-rate.



of the total—38 in 737, or 5 per cent. By calculation, 0.2 infective bites per person per year would produce just that parasite-index.

#### 5. *Discrepancy between Expectation and Observation*

In section IV, 4, we estimated that the *A. gambiae* found in Freetown houses from October, 1944, to March, 1946, would not infect more than one in 56 persons. But in section V, 3, it was shown that, in fact, one in four infants was infected. The discrepancy is considerable and may have three explanations: it may be that infants are bitten more frequently than adults; there may have been about 15 times more infected *A. gambiae* in Freetown than were found; or a large proportion of infections may have been acquired outside the city. Of the three, the last is the most likely explanation. This problem is dealt with more fully in the discussion.

#### 6. *Duration of Infection in Infants*

The duration of individual infections of *P. falciparum* is generally recognized as being from one to 12 months. It was hoped that the duration of single infections would be found by following up the infants after they were infected. The examination sequences of the infants who became infected are given in Table XI. Omissions make analysis difficult. Some infections appear to have been present at diagnosable levels for one month only. One typical clear-cut infection-history was shown by infant no. 123, who was negative for six months, and after being infected showed a progressive decline in parasite numbers for the next six months; thereafter he remained negative for 11 months. Infant no. 5 was continually positive for 11 months but appears to have been infected twice. Omitting infant no. 5, extracting those sequences which begin and end with a negative examination, and accepting omissions as positives, the mean period of infection of the 13 sequences so obtained is 3.15 months.

Some decline in severity of infection from the beginning of the sequences to the end is indicated.

#### 7. *Gametocyte Production*

Gametocytes were present on 13 occasions in 2,236 blood examinations, and in 17 per cent. of the positive observations. They appear to be produced for one month only, during which time trophozoite production is suppressed. The numbers of trophozoites relative to the number of gametes in the blood when gametes were present is given in Table VII. The mean number of trophozoites unaccompanied by gametes was 1,000 per 150 fields, while the mean number found accompanied by gametocytes was only 14.

#### 8. *The Temperature of the Infants*

Some 2,250 temperatures were taken. Temperatures of 99° F. (37.2° C.) or over were much more numerous during the course of infections than in uninfected infants. Yet some of the infections occurred without any detected rise of temperature above normal. The reactions are summarized as follows. Infants without plasmodia: temperatures of 99° F. or over seen eight times in 2,000 examinations. Infants with plasmodia: temperatures of 99° F. or over seen 35 times in 60 examinations; 100° F. (37.8° C.) or over seen six times in 60 examinations. No rise in temperature was noted in eight of 37 infected infants during the course of infection.

These temperature-reactions differ somewhat from those observed by Macdonald in 1926, when infants would be infected very much more frequently. It is suggested that, under the conditions then prevailing, they would rapidly acquire 'immunity' to infections. Parasites would sometimes be scarce in the blood and temperature-reactions slight but wide-spread. In 1945 'immunity' would be very slowly acquired, parasites would be more numerous in the blood, and temperature-reactions would be more severe and more often associated with active infection.

### 9. *Severity of Infections in Infants*

In this investigation positive observations are few, but they are nevertheless worth considering. The mean number of trophozoites seen in age-groups of three months, including all positive findings up to the age of 15 months, are given in Table VIII. Total positive findings are given in Table XI. The mean number of trophozoites in the age-group 10-12 months (Table VIII) is heavily overweighted by the inclusion of one abnormal observation—that of an infant who was seriously ill and who had 1,000 trophozoites per 100 leucocytes. These figures suggest that, contrary to what would be expected, the intensity of infection, as measured by the number of trophozoites in the blood, increases with age, at least up to the age of 14 or 15 months. Little reliance can be given to the age-group 0-3 months, and none to those over 15 months, since the investigation was abruptly stopped in June, 1946. The explanation appears to be that, although individual infections may start with high parasite counts and tend to fall to low levels at the end of the infection, those acquired by the younger children start and finish at a relatively lower level than infections acquired by the older age-groups. This series is interesting, in that it is not complicated by superimposed infections. Barber and Olinger (1931) also noted a rising intensity of infection with age in Nigerian infants.

Apparently the infants become increasingly susceptible as they grow older. Fig. 6 attempts to portray the change in parasite quantity with age. Have these infants some mechanism which protects them from infection at birth, and do they gradually become increasingly susceptible as they grow older? Other workers who have observed malaria in African infants have commented on 'immunity.' Their observations are summarized in the next section.

## VI. PREVIOUS WORK ON AFRICAN INFANTS

In 1925 Blacklock and Gordon (1925*b*) examined nearly 200 Freetown infants under one month old, and found only one—a child between three and four weeks old—infected with malaria. They wrote :

'This freedom from parasites in the peripheral blood may be due to freedom of the child from infection. If this is so, it may merely result from the fact that, for some unexplained reason, children up to a week or two old are little exposed to the bites of infected anophelines. On the other hand, it would be compatible with either a temporary general immunity, i.e., a condition during the existence of which the child is totally incapable of developing infection anywhere, or a condition of local immunity with partial tolerance, i.e., a condition in which the child is in fact infected, but in which the infection does not appear equally distributed throughout the body, but only in certain parts, of which the peripheral circulation is not one. . . .

'It seems not only legitimate but necessary to believe that in pregnant native women infected with malaria, there are certain portions of the circulatory system which are immune from infection ; while at the same time, in the same individuals, other portions, far from being immune, exhibit massive infection, accompanied by active sporulation. If we admit a local immunity in the case of the mother, it must be admitted that a similar condition may exist in the child.'

Blacklock and Gordon examined a selected series of children from the infant welfare clinic, and drew a curve of increase in the parasite-index with age. Of this curve they state :

'The character of this curve could be explained in either of two ways. It would be compatible with the idea that the child at birth was endowed with a passive immunity derived from the mother which steadily diminished until the age of  $1\frac{1}{2}$ . It would also be compatible with the idea that effective exposure to infection increases steadily as the child grows older until it reaches the age of  $1\frac{1}{2}$ . The flattening of the curve between the age of  $1\frac{1}{2}$  and  $2\frac{1}{2}$  . . . would, if the passive immunity theory is accepted, almost certainly be due to the gradual acquirement of active immunity by the child.'

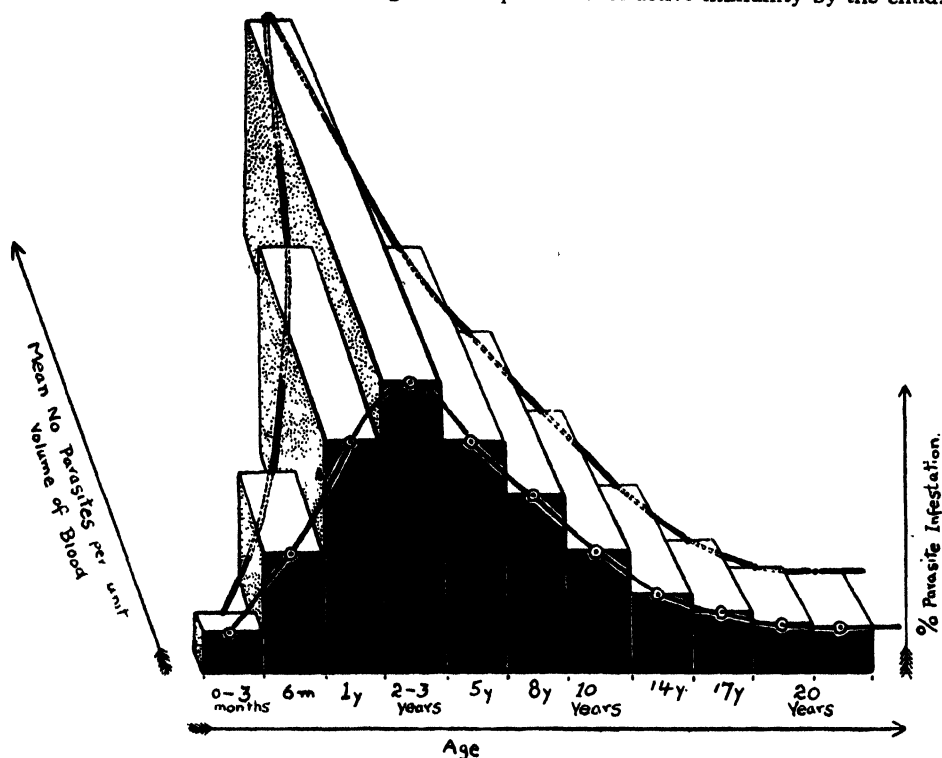


FIG. 6. Three-dimensional hypothetical representation of the variation of intensity of parasite infestation at different ages.

The horizontal axis shows age, increasing from left to right; the older age-groups are foreshortened. Based on the age-group scale the histogrammatic representation in the vertical axis depicts the change in percentage parasite infestation with age. Superimposed on this is the graphic representation of the same data. The depth of the receding columns represents the mean number of parasites per unit volume of blood (intensity) in each age-group, the scale being on a horizontal axis, at right angles to the axis depicting increasing age. The differences in the intensities of parasite infestation at different ages are greatly reduced; nevertheless the great variation in the degree of parasitization at different ages is quite clearly shown. The receding curve passing through the distal margins of the columns represents the change in parasite 'quantity' with age.

Barber and Olinger (1931) examined 3,176 infants attending clinics in Lagos and the suburb of Ebute Metta in Nigeria. The group is therefore selected, and is consequently not comparable with the Freetown infants investigated in 1944-46 or with those examined by Davey and Gordon in Freetown and Kissy in 1931. Barber and Olinger show that, whereas 90 per cent. of the infants were infected by one year, only 22 per cent. of infants three months old showed infections. They wrote :

'There is little evidence of a racial immunity against infection except possibly in the low infection index shown by infants 4 months old or younger. This index, only 22.2 per cent. in all infants, hardly exceeded 45 per cent. in Ebute Metta, a locality where infected *Anopheles* were especially abundant. One would expect in this locality a fairly large percentage of human infections after a residence of 4 days, and 4 weeks would seem to be ample for the infection of every infant. Infants may be better protected by clothing or night covering against the bites of mosquitos than are older children, but this explanation seems hardly adequate.'

Wilson (1936) studied infants in Tanganyika. He describes the contented healthy appearance of the African infants before infection with malaria, and says that 'It can . . . be stated that all cases examined within the first month (except in the period immediately after the rains) were negative.' He goes on to describe how, after infection, the health of the infants rapidly deteriorates and the haemoglobin values drop to nearly half the previous level\* ; and he states that by one year 87.5 per cent. were infected and by three years 100 per cent.

Davey and Gordon (1933) showed a striking deficiency in the infestation-rates in the age-group 0-3 months below the levels of infestation which would have been expected in view of the high rates observed in the older age-groups (see fig. 5 and Table IV).

Thomson (1935) examined 103 infants at monthly intervals in Nyasaland and found 70 per cent. infected in the first year and thereafter 100 per cent. He comments on the absence of infections in new-born infants.

Infants in Kenya were studied by Garnham (1935). The numbers examined were small, but the results caused the author to comment that up to the age of six months some infants escaped infection. While 100 per cent. were infected in the age-group 6-9 months, 37 per cent. were infected in the age-group 0-3 months.

Schwetz and Peel (1934), working at Stanleyville in the Belgian Congo, found 63 per cent. positive among 11 infants examined between the ages of eight and 30 days, 62.5 per cent. in 24 aged 1-3 months, and 94.6 per cent. in the age-group 6-12 months.

Eddey (1944), studying the effects of pyrethrum-spraying in the Gold Coast, examined infants both within and without the area of control. While 60 per cent. of infants in the uncontrolled area were infected in the age-group 0-3 months, 100 per cent. were infected by the age of one year in both controlled and uncontrolled areas.

To sum up, we can say that, varying with the locality, the observer, and the methods of blood examination and of selection of infants, the proportion of children infected in the first three months of life varies from 5 per cent. to 60 per cent., and that, by the age of one year, 70-100 per cent. are infected. These figures were obtained by a single blood examination. Repeated blood examinations of the same individual infants would probably show that a parasite-index of 70 per cent. is equivalent to 100 per cent., since parasites are not always detected in the blood during the course of infection by single examinations. Almost without exception observers who have examined African infants have commented on the low level of malaria infection in those under three months old. Even more striking is the failure of some workers to find infections in the first month of life. This might be understandable in Freetown in 1945, but its occurrence in widely separated parts of Africa, where malaria is intense, can only prove either that, for some reason, very young infants are not bitten by anophelines to the same extent as older children, or that, when bitten by

\* Haemoglobin levels of children aged 3-5 months are normally nearly half the level found at birth, and those between the ages of three months and 10 years are normally lower than adult levels (Hutchison and Hunter, 1940 ; Russell *et al.*, 1946).

infective mosquitoes, they possess some mechanism which prevents the appearance of plasmodia in the peripheral blood.

In the next section we will attempt to look a little deeper into this most interesting problem.

## VII. DISCUSSION

### 1. *Necessity for Caution in the Interpretation of Malaria Findings in Infants*

In discussing the transmission of malaria in infants, and in generalizing on what we learn from the examination of one-seventh of a cubic millimetre of their blood, we are still in the realms of surmise and analogy. Nothing is known of the biting-habits of *Anopheles gambiae* in relation to African infants, and we are therefore not entitled to apply what is otherwise known of them to the problem of malaria in children and adults. We can, however, attempt to clarify the problem of malaria transmission in the case of the children themselves. A large amount of work still remains to be done before any information which may be derived from the study of malaria in infants can safely lead to any generalizations. The present discussion merely attempts to clarify some of the obscure points and to bridge some of the more important gaps in our knowledge.

### 2. *Congenital Malaria*

When man is bitten by an anopheline mosquito with infective sporozoites of *P. falciparum* in its salivary glands, the intra-erythrocytic forms of the parasite are usually detectable on about the 10th day following the bite. Discovery of the parasite in the blood of infants under 10 days old therefore justifies our saying that they are derived from the mother. Before discussing the problem of infections derived from mosquito-bites, the extent of congenitally acquired infections must be known. The subject of congenital malaria in African infants has been studied by Blacklock and Gordon (1925*a*, 1925*b*), Mackay (1933), Schwetz and Peel (1934), Van den Branden (1927), Lombart (1931), Langeron (1927), Langeron and Van Nitsen (1927), and Valcke (1927). Mackay reported finding seven infections among 22 infants examined at birth, and Schwetz found infections in 3.6 per cent.

Between March, 1944, and March, 1947, the blood of African infants born of mothers admitted to the maternity hospital in Freetown was taken immediately after birth. The blood slides were examined by the senior pathologist. During that time 2,154 infants were examined, and seven, or 0.32 per cent., were found to have malaria parasites in their blood. Some of the infections were quite heavy. In three cases the infants' blood showed a different parasite count from the blood of the placenta to which it was attached, as well as from that of the mother. In the remaining four positives the parasite counts of blood in infant, mother and placenta were similar.

Altogether the blood of well over 2,000 African infants has been examined immediately after birth, and less than 1 per cent. was positive. It follows that, under normal conditions of malaria transmission, however intense, congenital malaria infections are rare in African infants, and we can therefore conclude that the majority acquire their malaria from infective mosquito-bites.

### 3. *Month-Old Infants*

There are relatively few records of blood examinations of African infants during the first month of life. Table IX contains the more important information. It appears that, depending upon the malariousness of a locality and upon the method of selecting the

infants and of examining the blood, infections can be demonstrated by a single examination in up to at least 50 per cent. of cases.

#### 4. *Fallacies in Parasite-Indices of Very Young Children*

Only Schwetz and Peel (1934) appear to have taken the incubation-period into consideration when estimating the proportion of infections among infants under a month old. These authors estimated the infection-index on infants from eight days to one month old. Eight days is the minimum incubation-period. It would appear that the safest procedure is to use the mean incubation-period, and to accept it as being 10 days. The parasite-index of infants under one month old would be obtained from the number examined over 10 days old, since those under 10 days old could not show infections caused by mosquito-bites, although infection occurred on the day they were born. In order to correct the subsequent infection-indices, however, it is still necessary to examine the blood of infants under 10 days old to discover the proportion with congenital infections. Otherwise, the proportion of infants infected under one month old may be relatively too low in comparison with older groups.

*P. malariae* is quite abundant in some parts of Africa. There is evidence (Barber and Olinger, 1931; Wilson, 1936) that this parasite, with its much longer extra-erythrocytic cycle, does not appear in the red blood-cells until between the second and fourth month of life and that it can thus produce a false contrast of parasite-indices between month-old infants and older children.

The parasite-index can also be given a false value in the younger age-groups if only 50, or even 100, fields of thick blood films are examined, rather than 150 or some larger number, for, as the severity of parasite infestation appears to rise with increasing age, the use of smaller blood samples will tend to show a proportion of infected infants below the true level—increasingly so the younger the age-group examined.

To continue the examination of infants from a period of low malaria transmission into one of high transmission would produce an abnormally high parasite-index in the older age-groups.

Moreover, should the infants live in an area where transmission is slower than in neighbouring areas, older children, being more likely to travel, will be exposed to conditions liable to produce infection-indices relatively too high in comparison with very young infants.

When all these factors are taken into consideration it is easy to see how the impression arises that fewer infections are found in very young children relative to the degree of infection noted later.

The infants studied in the present investigation can be used to illustrate the points raised. The percentage of positive infants in each age-group is plotted semi-logarithmically in fig. 7. The positives are based on the recognition of not less than four trophozoites in 150 fields of a thick blood film. The curve of increase of infection with age represents growth of infection uninfluenced by the effects of acquired immunity upon the course of the second infections, as in only one infant was a superimposed infection believed to have occurred. The curve is drawn through values calculated for the best-fitting curve

of the form  $\log y = \frac{-2.488684}{x} + 0.92429$ , where  $y$  = percentage of infections and  $x$  = age in months. It will be seen that the observed and calculated values for the proportion of infections in the age-group under three months are in very close agreement. There

were 500 observations in this group. The inference would be that infants up to a month old either escape infection or do not have parasites in their blood. Twenty-five infants under 11 days old were examined, and none was found to be positive; but allowance made for them in the 500 observations of children under three months old would scarcely alter the data. Since the rate of congenitally acquired infections in Freetown at that time was only 0.37 per cent., such allowance would only slightly increase the period of apparent freedom from infection.

On the other hand, there is no denying the fact that some infections were missed. Parasites were possibly present in some infants but were not detectable. Structures indis-

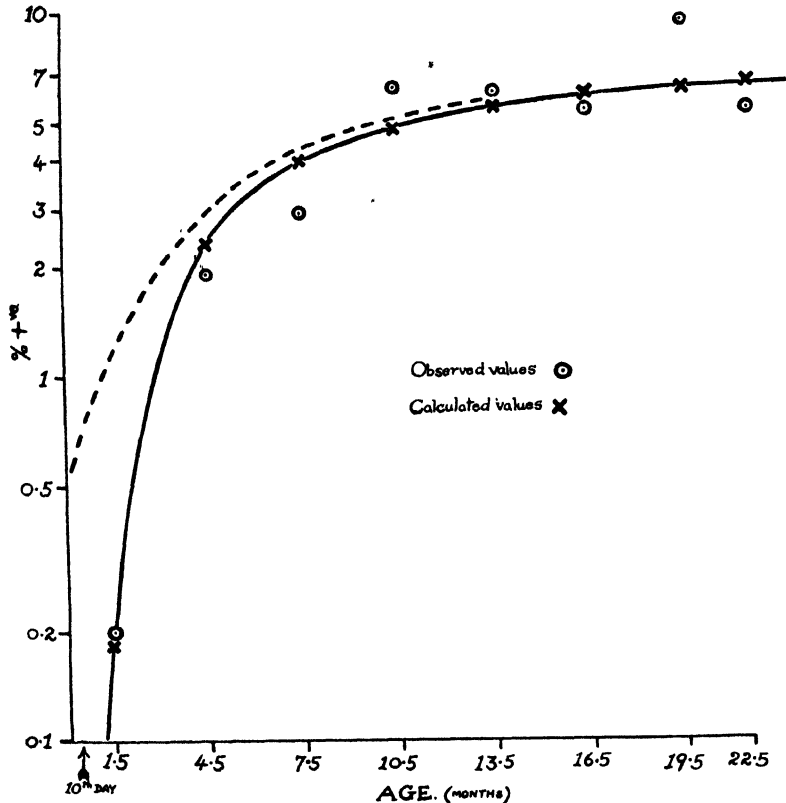


FIG. 7. Graph showing the rate of increase with age in percentage malaria infestation of infants. The black curve is based on the recognition of not less than four trophozoites; the dotted curve includes all positive findings, but the true curve of infestation is probably even higher, since some infections must have been missed.

tinguishable from parasites were seen in other cases but were not accepted, since they were too few for certain diagnosis. If all these parasites, or even some of them, were accepted, then there is no real justification for suggesting that infants either are not infected or do not show infections acquired from mosquito-bites during the first month of their lives.

Twelve slides showed less than four structures which were identified as trophozoites: five occurred in the first three months and four in the second three months of life. If these 12 are regarded as positive, they would, on inclusion with the other obvious positives,

raise the curve representing the parasite-rate (fig. 7) to the position of the dotted curve. This then passes through the incubation-period. There would then appear to be every reason to believe that African infants can be infected by mosquitoes from the day they are born, and that these infections can be identified in blood films after the 10th day.

### 5. *Immunity*

But there is no denying the existence of some factor which holds the multiplication of parasites in check or prevents large numbers from appearing in the blood, and which, by its mode of action, appears to resemble active acquired immunity.

Whatever brings about active proliferation of the reticulo-endothelial system of the mother might very well do the same to the unborn child. The child could be born with its reticulo-endothelial system already proliferated and ready to grapple with its first infection. Were the child not to be infected after birth this proliferated system would naturally resolve. This immunity might also be overcome by repeated infections of virulent parasitic strains.

### 6. *Accuracy of Anopheline Densities*

The discrepancy between the actual number of malaria infections acquired by the infants in Freetown and the expected number of infections (section V, 5) calls for comment. There is no reason for doubting the accuracy of the measure of anopheline abundance simply because more malaria was acquired by the infants than might be expected from the numbers and infectivity of the mosquitoes. The data on the anopheline populations are not exact, but they must be a close approximation, erring, if anything, on the high side. In the dry seasons, when anophelines seek the shelter of the houses from the hot dry conditions outside, the estimates of the numbers present were certainly too high. During the rains, control-measures cleared anophelines from the city, but quite high numbers appeared in small localized areas for short periods. In addition to the data derived from special catching-stations, a further mass of information was accumulated by spray-sheet catches made by the pyrethrum-spray gangs dispatched to exterminate these isolated anopheline outbreaks. The catches gave the gangs an idea of the limits of the area infested with *Anopheles*, and added an incentive to the workers, since they were able to see the results of their work. Comparison of their room-densities with the catching-station densities shows that, although they were working in areas known to be infested, lower figures were obtained. For example, the number of catching-station rooms examined between February and August, 1944, was 4,860, giving a room-density of 0.208 female *A. gambiae*. At the same time, the spray-gangs tested 4,000 rooms, obtaining a room-density figure of 0.164. The author's own impression is that the catching-station room-density figures more than compensate for any anophelines which could have escaped capture or were resting outside the houses. Muirhead Thomson (1947b) produces evidence which suggests that in Lagos nearly 30 per cent. of blood-fed *A. gambiae* normally leave houses at dawn (though some may re-enter later)—a figure which compares favourably with the observations made by Gordon *et al.* (1932) in Freetown.

### 7. *Unknown Factors in Malaria Transmission*

In attempting to find some relation between the number of infected anophelines in an area and the amount of malaria produced in the human population, some difficulties arise.



We do not know whether infants are more or less attractive than adult Africans to feeding *A. gambiae*, nor is it easy to follow the movements of Africans, or to learn where they acquire their infections.

Although it is usual to speak of mean numbers of anophelines per room, in practice an even distribution may never occur. While it is common to find a few rooms within a group of African houses which contain many *A. gambiae*, the majority contain relatively few. Moreover, some Africans appear to be more attractive than others to *A. gambiae* in search of a blood-meal, and this factor may be partly responsible for the uneven distribution in rooms; but the two factors combined probably result in some Africans being infected more frequently than others.

Not all infected bites by *A. gambiae* develop into human infections, but the proportion of those which do is unknown. Moreover, a few infected bites may be deviated to animals, such as goats.

Nor is it known what proportion of *A. gambiae* feeding in a room during the night actually remains in the room to be captured next day. Some may be resting out of doors.

Among the list of references at the end of this paper are many dealing with various aspects of these problems. From the information which they contain some rough idea of their effects on malaria transmission may be gathered.

### VIII. ESTIMATION OF MALARIA RISK

#### 1. Estimation of Infective Bites

As already outlined in previous sections of this paper, little is known of many factors in the chain of events involved in the transmission of malaria. Nevertheless, arbitrary values can be given to them and used in estimating approximate values of human parasite-indices from known *A. gambiae* populations. The attempt is well worth while, however inaccurate, the arbitrary values may be, and further work can be directed at finding the correct values.

In Freetown the parasite-index of young children can be approximately estimated by first assuming that a minimum residual parasite-index of 5 per cent. in infants and 10 per cent. in older children\* is acquired through immigration and visits to the surrounding uncontrolled areas (Turner and Walton, 1946). The parasite-index due to malaria contracted in Freetown is then estimated from the following formula: number of infective

mosquito-bites per child per annum =  $\frac{0.7xy}{z}$ , where  $x$  = mean number of female *A. gambiae* per room,  $y$  = mean figure of percentage sporozoite-rate, and  $z$  = mean number of persons per room. This abbreviated formula is derived from the following argument, which hinges on malaria transmission in an average room.

Suppose that an *A. gambiae* population exists in relation to an assumed static human population, and that the mean number of female *A. gambiae* per room is  $x$ . This can be expressed as  $365x$  female *A. gambiae* per average room per year. To make allowance for the uneven distribution of the insects and for the unequal attractiveness of different persons to them when feeding, a random distribution of the mosquitoes in the rooms is assumed and interpreted as reducing the effective number of *A. gambiae* by  $2/5$ .

If  $y$  per cent. of the mosquitoes are infected in the salivary glands, then  $\frac{365x \times 3}{5}$

\* In 1946 the parasite-index of 960 school-children, with a mean age of  $7\frac{1}{2}$  years, living in urban Freetown was 9.2 per cent. In that year 1,620 school-children of that age were examined in the whole city, and the parasite-index was 12.2 per cent. I regard this urban figure as about the lowest level of infection that could be achieved in Freetown by mosquito control.

is multiplied by  $y/100$ . This is divided by 2, to make allowance for *A. gambiae* feeding every other night, and reduced by  $1/3$ , to allow for the difference between infected and infective bites and bites deviated to animals. This gives the number of infective bites per room per year. Since the room contains average persons, the resulting number of infective bites is then divided by  $z$ , the mean number of persons per room, which gives the mean number of infective bites per person per year.

## 2. Estimation of Parasite-Index from the Number of Infective Bites

Assume that mosquito-bites are distributed at random. Then, if  $m$  be the number of infective bites per person per year, the number of persons bitten 0, 1, 2, 3, etc., times per year will be in the proportion of the Poisson series:  $e^{-m}(1, m, m^2/2!, \dots)$ .

Now if  $q$  be the mean proportion of the year for which an infected susceptible person shows parasites, and  $p$  the proportion in which none is shown (i.e.,  $p + q = 1$ ), then, at any one examination of susceptible persons, of those bitten once a proportion  $p$  will show no parasites. If the infections are independent, of those bitten twice a proportion  $p^2$  will show no parasites; of those bitten three times a proportion  $p^3$ ; and so on. Hence the proportion of all susceptible persons showing no parasites will be:  $e^{-m}(1 + pm + p^2m^2/2! + p^3m^3/3! + \dots) = e^{-m}e^{pm} = e^{-m}(1 - p) = e^{-mq}$ .

Therefore the percentage of susceptible persons showing parasites at one examination will be  $100(1 - e^{-mq})$ . Using the value  $q = 0.26$  (section V, 6), which is the best estimate available, the values of the percentage of susceptible persons showing parasites have been calculated for various values of  $m$  (Table VI).

After the approximate number of infective bites per year has been estimated, the corresponding parasite-index is obtained from the table. In Freetown 5 per cent. is added in the case of infants and 10 per cent. in the case of older children. In order to test this theoretical distribution of the number of infective bites per person per year, the parasite-index observed at various times in Freetown and the neighbouring town of Kissy was compared with that calculated from the anopheline density and sporozoite-rates. The data are given in Table X, from which it is seen that agreement is very close.

## IX. TABULATED DATA

TABLE I  
Infant-mortality rates

	Jan.	Feb.	March	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Mean annual rate
Mean rates													
1936-43 ...	159	175	159	189	224	223	223	245	187	168	162	162	189
1944 ...	140	127	164	165	157	172	137	151	132	152	164	177	153
1945 ...	144	186	134	141	130	199	148	171	186	207	169	169	165

The high rate for October, 1945, is due to smallpox.

TABLE II  
Number of female *A. gambiae* per 1,000 rooms per month in Freetown, June, 1944, to May, 1946

June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	Apr.	May
261	447	189	55	8	4	7	11	10	4	7	64
330	116	37	18	4	1	15	23	20	27	20	147

TABLE III  
Number of female *A. gambiae* per average Freetown room per season

1944		1945		1946
Wet Season	Dry Season	Wet Season	Dry Season	Wet Season
Apr.-Sept.	Oct.-March	Apr.-Sept.	Oct.-March	Apr.-Sept.
31.0	1.3	17.0	2.7	30.3

TABLE IV  
Percentage infestation in three-months age-groups

Age	0-3 months	4-6 months	7-9 months	10-12 months	2 years
Freetown, 1931 ...	4.8	41.2	58.8	70.6	83.7
" 1945 ...	0.2	1.9	2.8	6.2	c.6.5
Kissy, 1931 ...	51.6	84.0	89.0	92.2	100

TABLE V  
Parasite-index of infants in the same age-groups in different seasons

Age	Dry season 1944-45	Wet season 1945	Dry season 1945-46	Wet season 1946
4-10 months	3.4% ; 432	2.7% ; 334	1.5% ; 132	—
1-16 "	—	4.7% ; 682	2.8% ; 352	1.7% ; 60
11-22 "	—	—	5.0% ; 352	5.7% ; 139

TABLE VI  
Parasite-indices produced in a group of young children by various numbers of infective mosquito-bites received in a year

No. of infective bites per child per year	Calculated parasite-rate per cent. in young children
0.01	0.26
0.10	2.57
0.20	5.07
0.30	7.50
0.40	9.90
0.50	12.20
0.70	16.60
1.00	22.90
1.50	32.30
2.00	40.50
3.00	54.20
4.00	64.70
5.00	72.70
7.00	83.80
10.00	92.60
20.00	99.45
30.00	99.96
50.00	99.99

TABLE VII  
Number of trophozoites associated with gametes

No. of trophozoites ...	0	0	0	0	0	0	0	0	1	1	3	5	59
No. of gametes...	1	1	2	2	3	6	18	52	2	2	12	1	8

TABLE VIII  
Intensity of infection in relation to age of infant

Age-group, in months ...	0-3	4-6	7-9	10-12	13-15
Mean no. of trophozoites ...	Under 5	246	290	1,370	1,400

TABLE IX  
Malaria infection in month-old African infants

Author and locality	No. examined	Percentage positive	Congenital infections
Schwetz ; Belgian Congo ...	11	63	3.6%
Barber ; Nigeria ...	16	25	—
Freetown, 1944-46 : clinic infants ...	493	7.9	0.32%
Blacklock ; Freetown, 1925 : clinic infants	196	1.0	0%
Freetown, 1945 : present series ...	148	0	0.32%

TABLE X  
Comparison between estimated and observed parasite-index

Group of persons examined	Anopheline density, as measured	Sporozoite-rate percentage, as measured	Calculated no. of infected bites per person per year (from columns 2 and 3)	Estimated parasite-index percentage (from Table VI)	Corrected parasite-index percentage (see section VIII, 1)	Observed parasite-index percentage
Freetown infants, 1931 (Davey)	1.6 (estimated) (see section V, 2)	10	5.6	75	80	83.7
Freetown infants, 1945 period	0.05	0.42	0.0073	0.2	5.2	6
Kissy infants, 1931 (Davey)	15.2	7	37.5	99.9	100	92.2
Freetown urban children, wet season 1944	0.17	4.8	0.29	7.5	17.5	18.3
Freetown urban children, wet season 1945	0.095	0.47 (falling)	0.016	0.5	10.5	15.2

TABLE  
Summary of monthly examinations

Serial no. of infant	Age of infant when										
	1	2	3	4	5	6	7	8	9	10	11
1	—	—	—	—	—	?	?	?	12	15	
5	?	?	?	—	—	1,000	35	115	75	16	2 G
10	?	?	—	—	—	—	?	—	—	?	—
12	—	—	—	—	?	?	—	—	—	—	—
13	?	?	—	—	—	—	?	?	—	75	2,250
21	—	?	—	—	—	—	—	—	—	—	—
40	?	?	—	—	—	?	?	—	?	?	—
51	—	—	—	—	?	—	—	?	—	—	—
69	?	?	?	—	—	—	—	?	—	—	—
72	—	—	—	—	?	—	—	—	—	—	—
78	?	?	?	?	—	?	—	?	—	—	—
82	?	?	?	—	—	—	?	—	—	—	—
84	—	—	—	—	?	—	—	?	—	—	—
85	?	?	?	?	—	?	?	—	25	1+2 G	?
96	—	—	—	—	?	—	—	—	—	—	—
97	—	—	—	—	?	?	—	—	—	—	—
100	—	—	—	—	?	—	—	—	—	—	?
123	—	—	—	—	—	—	2,000	?	150	85	20
134	?	—	—	—	?	—	—	?	?	—	—
135	?	—	—	?	—	—	—	?	?	—	—
142	—	—	—	—	?	?	350	—	—	—	—
145	—	—	—	—	—	—	?	—	—	—	—
148	?	?	?	—	—	?	?	—	—	—	—
156	?	—	—	—	—	—	?	—	—	15,000	750
159	—	—	—	?	58+8 G	60	—	—	—	—	—
193	—	—	?	?	—	5	?	—	—	87	160
202	?	?	?	450	50	?	450	200	1 2 G	90	45
209	?	?	?	—	—	—	—	—	56	—	—
212	?	—	—	300	—	—	—	—	?	?	—
216	?	—	?	—	—	—	—	15	?	?	—
217	?	—	?	—	—	42	—	—	?	?	—

A dash (—) indicates that no plasmodia were found on examination.

A question-mark (?) indicates that the infant was temporarily missing and was not examined.

## X. SUMMARY

1. During the first part of the recent war, *Plasmodium falciparum* malaria became a serious menace to the convoy organization in the West African convoy-port of Freetown.

2. Malaria control became a necessity, and a reorganized meticulous antimosquito campaign was started in 1943 against the local malaria vector, *Anopheles gambiae* Giles, both in the city and in the neighbouring districts.

3. Antilarval measures were co-ordinated with extensive house-catches of adult mosquitoes and assisted by localized pyrethrum-spraying.

4. A dramatic reduction in the numbers of infected anophelines was achieved.

5. Methods are described of investigating malaria in African infants to measure quantitatively the effect of the control-measures upon the malaria infestation of the human population.

6. It is shown that the control achieved was virtually 100 per cent. effective in Freetown.

## XI

## of malaria-positive infants

examined, in months

12	13	14	15	16	17	18	19	20	21	22	23
6,000	2,500	450	450	—	—	—	—	—	?	1G	—
?	300	3,000	250	—	?	?	?	?	20	—	—
3 G	450	?	450	—	—	60	2G	?	1 G	—	—
21	—	—	7,500	1,500	?	?	?	—	—	—	—
—	—	—	—	—	—	?	?	—	?	—	—
—	?	—	—	?	?	35	12	—	—	—	—
—	—	—	—	—	—	?	?	54 G	—	—	—
?	?	—	?	1,500	—	?	?	—	—	25	0
—	—	—	—	?	?	—	?	1,500	20	—	—
8	—	—	—	—	—	60	3+12 G	—	—	—	—
—	—	—	—	?	?	—	?	?	3,000	—	—
—	—	—	—	?	?	—	?	?	300	—	—
8	63	18 G	30	?	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	30	—	—
—	—	—	—	750	450	—	10	—	—	( <i>P. malariae</i> )	—
?	—	—	—	—	?	—	?	?	60	—	—
?	450	—	—	?	8	—	?	40	—	—	—
5+1 G	—	—	—	—	—	—	—	—	—	—	—
39	—	—	—	—	—	—	—	—	—	—	—
?	—	—	—	—	—	—	—	—	—	—	—
?	60	—	—	—	—	—	—	—	—	—	—

Figures refer to the number of trophozoites found; a figure followed by the letter G indicates the number of gametocytes found.

7. By 1945 only 5-7 per cent. of African infants in Freetown were carrying malaria infections at the age of two years.

8. It is suggested that the majority of these infections were contracted from sources located outside the city.

9. A parasite-index in infants of 5 per cent. was found to result when one in every four infants was infected in 18 months.

10. Previous work on malaria in African infants is reviewed, and shows that, in widely separated parts of Africa where *A. gambiae* was a malaria vector, 100 per cent. of the infants had infections by the age of 2-3 years.

11. Parasite-rates of African infants and children were estimated from observed anopheline abundance and infection-rates, and were confirmed by blood examination.

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